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LAW OFFICES

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09/674379

October 30, 2000

ROX PCT

Assistant Commissioner for Patents Washington, D.C. 20231

PCT/PCT/JP99/02284 -filed April 28, 1999

Re:

Application of Tasuku HONJO, Kei TASHIRO and Tomoyuki NAKAMURA A NOVEL POLYPEPTIDE, A cDNA ENCODING THE POLYPEPTIDE AND UTILIZATION THEREOF Our Ref: Q61531

Dear Sir:

The following documents and fees are submitted herewith in connection with the above application for the purpose of entering the National stage under 35 U.S.C. § 371 and in accordance with Chapter II of the Patent Cooperation Treaty:

- ☑ an English translation of the International Application.
- ☑ two (2) sheet(s) of informal drawings.
- ☑ Notification Concerning Submission or Transmittal of Priority Document w/faithful translation
- ☑ thirty-eight (38) pages of Sequence Listing and a 3.5"disk containing Sequence Listing.
- ☑ Statement in Support of Submission in Accordance with 37 C.F.R. § 1.821.
- International Preliminary Examination Report
- International Search Report, Information Disclosure Statement and Form PTO-1449.
- ☑ Preliminary Amendment

The Declaration and Power of Attorney and Assignment, will be submitted at a later date.

It is assumed that copies of the International Application, the International Search Report, the International Preliminary Examination Report, and any Articles 19 and 34 amendments as required by § 371(c) will be supplied directly by the International Bureau, but if further copies are needed, the undersigned can easily provide them upon request.

The Government filing fee is calculated as follows:

Total claims Independent claims	19	-	20 3	=	 X X	\$18.00 \$80.00	= -	\$.00 \$.00
Base Fee							_	\$860.00
Multiple Dependent Claim Fe	ee						-	\$270.00

TOTAL FEE

\$1130.00

Kg/lo 32,765

A check for the statutory filing fee of \$1130.00 is attached. You are also directed and authorized to charge or credit any difference or overpayment to said Account. The Commissioner is hereby authorized to charge any fees under 37 C.F.R. §§ 1.16, 1.17 and 1.492 which may be required during the entire pendency of the application to Deposit Account No. 19-4880. A duplicate copy of this transmittal letter is attached.

Priority is claimed from April 28, 1998 based on Japanese Application No. Hei. 10-119731.

Since October 28, 2000 (30 months from the priority date) fell on a Saturday, the submission of these papers on Monday, October 30, 2000, is sufficient for entry of National Stage of the above application.

Mark Boland

MXB/amt



PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Tasuku HONJO, et al.

Appln. No.: 09/674,379

Confirmation No.: Not Yet Assigned

Filed: October 30, 2000

A NOVEL POLYPEPTIDE, A CDNA ENCODING THE POLYPEPTIDE AND UTILIZATION THEREOF

Group Art Unit: Not Yet Assigned

Examiner: Not Yet Assigned

PRELIMINARY AMENDMENT

Commissioner for Patents Washington, D.C. 20231

Sir:

For:

Prior to examination, please amend the above-identified application as follows:

IN THE CLAIMS:

Please add the following new claims:

- 14. (new)A method for promoting smooth muscle growth or vasculogenesis which comprises administering to a mammalian subject a therapeutically effective amount of an antibody of claim 9.
- 15. (new)A pharmaceutical composition for promoting smooth muscle cell growth or vasculogenesis which comprises administering to a mammalian subject a therapeutically effective amount of an antibody of claim 9.

REMARKS

Entry and consideration of this Amendment is respectfully requested.

Respectfully submitted,

SUGHRUE MION, PLLC 2100 Pennsylvania Avenue, N.W.

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MXB/amt

Date: December 10, 2001

Registration No. 32,197

APPENDIX

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS:

Claims 14 and 15 are added as new claims.

09/674379

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Tasuku HONJO, et al.

Appln. No.: Not Yet Assigned

Filed: October 30, 2000

For:

UTILIZATION THEREOF

Group Art Unit: Not Yet Assigned

Examiner: Not Yet Assigned

A NOVEL POLYPEPTIDE, A cDNA ENCODING THE POLYPEPTIDE AND

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents Washington, D.C. 20231

Sir:

Prior to examination, please amend the above-identified application as follows:

IN THE SPECIFICATION:

Page 4, line 25, please delete "1, 4, 6, 9, 11 or 14," and insert --3, 4, 8, 9, 13 or 14,--

Page 4, line 27, please delete "2, 5, 7, 10," and insert --1, 5, 6, 10,--

Page 4, line 28, please delete "12 or 15," and insert --11 or 15,--

Page 5, line 2, please delete "3, 8 or 13" and insert --2, 7 or 12--

Page 5, line 10, please delete "1, 4, 6, 9, 11 or 14" and insert --3, 4, 8, 9, 13 or 14--

Page 5, line 15, please delete "2, 5, 7, 10, 12 or" and insert --1, 5, 6, 10, 11 or--

Page 5, line 17, please delete "2, 5, 7, 10," and insert --1, 5, 7, 10,--

Page 5, line 18, please delete "12 or 15" and insert --11 or 15--

Page 5, line 21, after "SEQ ID NO." please delete "1" and insert --3--

Page 5, line 22, please delete "4, 6, 9, 11 or 14" and insert --4, 8, 9, 13 or 14--

Page 5, line 24, please delete "1, 4," and insert --3, 4,--

Page 5, line 25, please delete "6, 9, 11 or 14" and insert --8, 9, 13 or 14--

Page 6, line 1, please delete "1, 4, 6, 9, 11 or 14" and insert --3, 4, 8, 9, 13 or 14--

PRELIMINARY AMENDMENT

Q61531

- Page 6, line 3, after "SEQ ID NO." please delete "1" and insert --3--
- Page 6, line 7, please delete "1, 4, 6, 9, 11 or 14" and insert --3, 4, 8, 9, 13 or 14--
- Page 6, line 12, please delete "2, 5, 7, 10, 12 or 15" and insert --1, 5, 6, 10, 11 or 15--
- Page 6, line 14, please delete "2, 5, 7, 10, 12 or 15" and insert --1, 5, 6, 10, 11 or 15--
- Page 6, line 19, please delete "2, 5, 7, 10, 12 or 15" and insert --1, 5, 6, 10, 11 or 15--
- Page 7, line 4, after "SEQ ID NO." please delete "2" and insert --1--
- Page 7, line 5, please delete "3, 5, 7, 8, 10, 12, 13 or 15" and insert --2, 5, 6, 8, 10, 11, 12 or 15--
 - Page 8, line 4, after "SEQ ID NO." please delete "1" and insert --3--
 - Page 8, line 8, please delete "1, 4, 6, 9, 11 or" and insert --3, 4, 8, 9, 13 or--
 - Page 8, line 17, please delete "1, 4, 6, 9, 11 or 14" and insert --3, 4, 8, 9, 13 or 14--
 - Page 8, line 23, please delete "3, 8 or 13" and insert --2, 7 or 12--
 - Page 11, line 19, please delete "2, 5, 7, 10, 12 or" and insert --1, 5, 6, 10, 11 or--
 - Page 12, line 9, please delete "2, 7 or 12" and insert --1, 6 or 11--
 - Page 12, line 10, please delete "3, 8 or 13" and insert --2, 7 or 12--
 - Page 12, line 13, please delete "2, 5, 7, 10, 12" and insert --1, 5, 6, 10, 11--
 - Page 13, line 14, please delete "2, 5, 7, 10, 12 or 15" and insert --1, 5, 6, 10, 11 or 15--
 - Page 33, line 23, after "SEQ ID NO." please delete "3" and insert --2--
 - Page 33, line 25, after "SEO ID NO." please delete "2" and insert --1--
 - Page 33, line 26, after "SEQ ID NO." please delete "1" and insert --3--
 - Page 33, line 27, after "SEQ ID NO." please delete "3" and insert --2--
 - Page 35, line 9, after "SEQ ID NO." please delete "3" and insert --2--
 - Page 35, line 11, after "NO." please delete "7 and 8" and insert --6 and 7--
 - Page 35, line 14, after "SEQ ID NO." please delete "8" and insert --7--
 - Page 35, line 16, after "in SEQ ID NO." please delete "6" and insert --8--
 - Page 35, line 16, after "of SEO ID NO." please delete "1" and insert --3--
 - Page 35, line 17, after "SEQ ID NO." please delete "6" and insert --8--
 - Page 35, line 19, after "SEQ ID NO." please delete "8" and insert --7--
 - Page 36, line 5, before "was determined" please delete "13" and insert --12--
 - Page 36, line 7, after "NO." please delete "12" and insert --11--
 - Page 36, line 7, after "in SEQ ID NO." please delete "11" and insert --13--

PRELIMINARY AMENDMENT

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Page 36, line 14, after "SEQ ID NO." please delete "13" and insert --12--

Page 36, line 24, after "SEQ ID NO." please delete "3" and insert --2--

Page 38, line 17, after "SEQ ID NO." please delete "13" and insert --12--

Page 39, line 11, after "SEQ ID NO." please delete "13" and insert --12--

Page 40, line 14, after "SEQ ID NO." please delete "1" and insert --3--

Page 40, line 15, after "SEQ ID NO." please delete "1" and insert --3--

Page 40, line 16, after "SEQ ID NO." please delete "1" and insert --3--

IN THE CLAIMS:

Claim 1, line 2, after "SEQ ID NO." please delete "11" and insert --13--

Claim 2, line 2, after "SEQ ID NO." please delete "11" and insert --13--

Claim 4, line 2, after "SEQ ID NO." please delete "12" and insert --11--

Claim 5, line 2, after "SEQ ID NO." please delete "13" and insert --12--

REMARKS

The amendments to the specification and claims are necessitated by the revision of the Sequence Listing.

The Sequence Listing was originally prepared using PatentIn version 2.0. Due to the requirement that Sequence Listings now be prepared using PatentIn version 2.1 or 3.0, a new Sequence Listing was prepared. The order of the sequences prepared using the new Sequence Listing was altered, as compared to the Sequence Listing prepared using PatentIn version 2.0. Therefore, the specification and claims have been amended to match the updated Sequence Listing. Accordingly, no new matter has been introduced.

PRELIMINARY AMENDMENT

Q61531

Entry and consideration of this Amendment is respectfully requested.

Respectfully submitted,

Mark A. Hissong

Registration No. 44,765

SUGHRUE, MION, ZINN, MACPEAK & SEAS, PLLC 2100 Pennsylvania Avenue, N.W. Washington, D.C. 20037-3213 Telephone: (202) 293-7060 Facsimile: (202) 293-7860

Date: October 30, 2000

2/PRTS

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Specification

A novel polypeptide, a cDNA encoding the polypeptide and utilization thereof

Field of the Invention

The present invention provides a novel polypeptide, a cDNA encoding the polypeptide, and utilization thereof.

Background of the Invention

In modern medical research, cardiovascular biology is a field that attracts considerable attention because cardiovascular disease is the leading cause of mortality. Cardiovascular research has revealed important facts about neointimal formation and arterial remodeling, both of which are thought to contribute to plaque formation in atherosclerosis and blood vessel narrowing. For example, there are three aspects of the cellular process in hypercholesterolaemia induced blood vessel damage in animal models that mimic human development of arteriosclerotic coronary disease. The three elements that form lesions on the artery wall are: a) proliferation of smooth muscle cells, macrophages and lymphocytes, b) formation of connective tissues (mainly elastic fiber proteins, collagen and proteoglycans made by smooth muscle cells in a process similar to scar formation), and c) the accumulation of lipid and cholesterol in the newly formed connective tissue matrices. The exact sequence of the three damaging elements are debatable, but it is clear that the abnormal dedifferentiation, redifferentiation and growth of smooth muscle cells contribute structurally to vessel damage. Moreover, another significant pathological process that involves abnormal smooth muscle cell growth is restenosis after Percutaneous transluminal coronary angioplasty (PTCA).

The present inventors made reasonable efforts, by isolation of the

molecules related to participation of smooth muscle cells in angiogenesis, for the aim to utilize them for regulation of abnormal proliferation of smooth muscle cells such like described above.

In order to obtain a certain polypeptide or cDNA coding for the same, there has been generally employed a method composed of detecting the aimed biological activity in a tissue or a cell culture medium, then identifying a polypeptide as substance of the activity through the isolation and purification and isolating a gene encoding the polypeptide or expression-cloning method to isolate a gene by access of the biological activity of the polypeptide encoded by it.

Because in many cases, however, physiologically active polypeptides have various biological activities, when taking the method to approaches based on a certain activity to isolate a gene, it has increasingly been happened that the gene is turned out to be identical to a known gene which has another activity after spending much efforts to isolate it. And because, in many cases, biological factors are produced only in a very slight amount or only in a specific condition, it is often made difficult to isolate and purify a factor and detect its biological activity.

Recent rapid developments in techniques for constructing cDNAs and sequencing techniques have made it possible to quickly sequence a large amount of cDNAs. By utilizing these techniques, a process, which comprises constructing cDNAs at random, identifying the nucleotide sequences thereof, expressing novel polypeptides encoded by them, is now in progress. Although this process is advantageous in that a gene can be cloned and information regarding its nucleotide sequence can be obtained without any biochemical or genetic analysis, the target gene can be discovered thereby only accidentally in many cases.

Disclosure of the Invention

The present inventors investigated to find novel factors (polypeptides) which are useful for study or for the treatment or diagnosis of diseases induced by abnormal proliferation of smooth muscle. Especially, we had aimed secreted proteins and membrane proteins which have signal sequences for secretion.

The present inventors have studied cloning method of genes coding proliferation and/or differentiation factors functioning in hematopoietic systems and immune systems. Focusing their attention on the fact that most of the secretory proteins such as proliferation and/or differentiation factors (for example various cytokines) and membrane proteins such as receptors thereof (hereafter these proteins will be referred to generally as secretory proteins and the like) have sequences called signal peptides in the N-termini, the inventors conducted extensive studies on a process for efficiently and selectively cloning a gene coding for a signal peptide. Finally, we have successfully invented a screening method for cDNAs having sequence encoding signal peptides, we called the method as signal sequence trap (SST) (Japanese Patent Publication No. 6-315380).

We also developed yeast SST method on the same concept. By the method using yeast, genes including sequence encoding signal peptide can be identified more easily and effectively (USP No. 5,536,637).

By using the present method, the present inventors identified novel secreted protein produced by mouse embryonic heart and human kidney and a cDNA fragments encoding them, and by using the sequence information of the cDNA fragments they isolated each full-length cDNA from mouse embryonic heart and human kidney. And they discovered that the polypeptides had functions to suppress smooth muscle cells.

The present cDNA sequence was named as a clone mouse A55 and isolated

from cDNA library derived from mouse embryonic heart based on genetic information obtained by using the Yeast SST method described above. The clone, mouse A55 is a full-length cDNA encoding a secreted polypeptide (which is called mouse A55 polypeptide here).

The present cDNA sequence was named as a clone human A55 and isolated from cDNA library derived from human brain based on genetic information obtained from human kidney by using the Yeast SST method described above. The clone, human A55 is a full-length cDNA encoding a secreted polypeptide (which is called human A55 polypeptide here).

There was no DNA sequence which is identical to that of mouse and human A55 of the present invention, when DNA sequence of mouse and human A55 were compared with data base by BLASTN and FASTA. And there was no polypeptides which is identical to that of mouse and human A55 of the present invention, when amino acid sequence of mouse and human A55 was compared with data base by BLASTX, BLASTP and FASTA. So the polypeptides of the present invention are considered to be novel.

The inventors discovered that the polypeptides had functions to suppress smooth muscle cells. Accordingly, the polypeptides may be useful for treatment of diseases related to abnormal proliferation of smooth muscle cells, for example, arteriosclerotic coronary disease, neointimal formation which results in restenosis after percutaneous transluminal coronary angioplasty and myosarcoma.

The present invention provides:

- a polypeptide comprising an amino acid sequence shown in SEQ ID NO. 1, 4, 6, 9, 11 or 14,
- a cDNA encoding the polypeptide described above (1),
- a cDNA having an nucleotide sequence shown in SEQ ID NO. 2, 5, 7, 10, 12 or 15,

a cDNA that consists of an nucleotide sequence shown in SEQ ID NO. 3, 8 or 13.

Brief Description of Figures

- Fig. 1 It shows that mouse A55 protein inhibits proliferation of rat aortic vascular smooth muscle cells which was stimulated by PDGF.
- Fig. 2 It shows that human A55 protein inhibits proliferation of rat aortic vascular smooth muscle cells which was stimulated by PDGF.

Detailed Discliption

The present invention is concerned with a polypeptide that comprising the amino acid sequence shown in SEQ ID NO. 1, 4, 6, 9, 11 or 14 in substantially purified form, a homologue thereof, a fragment of the sequence and a homologue of the fragment.

Further, the present invention is concerned with a cDNA encoding the above peptides. More particularly the present invention is provided cDNA comprising the nucleotide sequence shown in SEQ ID NO. 2, 5, 7, 10, 12 or 15, and cDNA containing a fragment which is selectively hybridizing to the cDNA that comprising nucleotide sequence shown in SEQ ID NO. 2, 5, 7, 10, 12 or 15. Complementary sequence of the above nucleotide sequence is also included in cDNA selectively hybridized. Hybridization are performed in an stringent condition.

A polypeptide comprising amino acid sequence shown in SEQ ID NO. 1, 4, 6, 9, 11 or 14 in substantially purified form will generally comprise the polypeptide in a preparation in which more than 90%, e.g. 95%, 98% or 99% of the polypeptide in the preparation is that of the SEQ ID NO. 1, 4, 6, 9, 11 or 14.

A homologue of polypeptide comprising amino acid sequence shown in

SEQ ID NO. 1, 4, 6, 9, 11 or 14 will be generally at least 70%, preferably at least 80 or 90% and more preferably at least 95% homologous to the polypeptide of SEQ ID NO. 1 over a region of at least 20, preferably at least 30, for instance 40, 60 or 100 more contiguous amino acids. Such a polypeptide homologue will be referred to a polypeptide of the present invention.

Generally, a fragment of polypeptide comprising amino acid sequence shown in SEQ ID NO. 1, 4, 6, 9, 11 or 14 or its homologues will be at least 10, preferably at least 15, for example 20, 25, 30, 40, 50 or 60 amino acids in length, and are also referred to by the term "a polypeptide of the present invention".

A cDNA capable of selectively hybridizing to the cDNA comprising nucleotide sequence shown in SEQ ID NO. 2, 5, 7, 10, 12 or 15 will be generally at least 70%, preferably at least 80 or 90% and more preferably at least 95% homologous to the cDNA of SEQ ID NO. 2, 5, 7, 10, 12 or 15 over a region of at least 20, preferably at least 30, for instance 40, 60 or 100 or more contiguous nucleotides. Such cDNA will be referred to "a cDNA of the present invention".

Fragments of the cDNA comprising nucleotide sequence shown in SEQ ID NO. 2, 5, 7, 10, 12 or 15 will be at least 10, preferably at least 15, for example 20, 25, 30 or 40 nucleotides in length, and will be also referred to "a cDNA of the present invention" as used herein.

A further embodiment of the present invention provides replication and expression vectors carrying cDNA of the invention. The vectors may be, for example, plasmid, virus or phage vectors provided with an origin of replication, optionally a promoter for the expression of the said cDNA and optionally a regulator of the promoter. The vector may contain one or more selectable marker genes, for example a ampicillin resistance gene. The vector may be used in vitro, for example of the production of RNA corresponding

to the cDNA, or used to transfect or transfect a host cell.

A further embodiment of the present invention provides host cells transformed with the vectors for the replication and expression of the cDNA of the invention, including the nucleotide sequence shown in SEQ ID NO. 2, 3, 5, 7, 8, 10, 12, 13 or 15 or the open reading frame thereof. The cells will be chosen to be compatible with the vector and may for example be bacterial, yeast, insect or mammalian.

A further embodiment of the present invention provides a method of producing a polypeptide which comprises culturing host cells of the present invention under conditions effective to express a polypeptide of the invention. Preferably, in addition, such a method is carried out under conditions in which the polypeptide of the invention is expressed and then produced from the host cells.

cDNA of the present invention may also be inserted into the vectors described above in an antisense orientation in order to proved for the production of antisense RNA. Such antisense RNA may be used in a method of controlling the levels of a polypeptide of the invention in a cell.

The invention also provides monoclonal or polyclonal antibodies against a polypeptide of the invention. The invention further provides a process for the production of monoclonal or polyclonal antibodies to the polypeptides of the invention. Monoclonal antibodies may be prepared by common hybridoma technology using polypeptides of the invention or fragments thereof, as an immunogen. Polyclonal antibodies may also be prepared by common means which comprise inoculating host animals, for example a rat or a rabbit, with polypeptides of the invention and recovering immune serum.

The present invention also provides pharmaceutical compositions containing a polypeptide of the invention, or an antibody thereof, in association with a pharmaceutically acceptable diluent and/or carrier.

The polypeptide of the present invention includes that which a part of their amino acid sequence is lacking (e.g., a polypeptide comprised of the only essential sequence for revealing a biological activity in an amino acid sequence shown in SEQ ID NO.1), that which a part of their amino acid sequence is replaced by other amino acids (e.g., those replaced by an amino acid having a similar property) and that which other amino acids are added or inserted into a part of their amino acid sequence, as well as those comprising the amino acid sequence shown in SEQ ID NO. 1, 4, 6, 9, 11 or 14.

As known well, there are one to six kinds of codon as that encoding one amino acid (for example, one kind of codon for Methioine (Met), and six kinds of codon for leucine (Leu) are known). Accordingly, the nucleotide sequence of cDNA can be changed in order to encode the polypeptide having the same amino acid sequence.

The DNA of the present invention, specified in (2) includes a group of every nucleotide sequences encoding polypeptides (1) shown in SEQ ID NO. 1, 4, 6, 9, 11 or 14. There is a probability that yield of a polypeptide is improved by changing a nucleotide sequence.

The cDNA specified in (3) is the embodiment of the cDNA shown in (2), and indicate the sequence of natural form.

The cDNA shown in (4) indicates the sequence of the cDNA specified in (3) with natural non-translational region.

cDNA carrying nucleotide sequence shown in SEQ ID NO. 3, 8 or 13 is prepared by the following method:

Brief description of Yeast SST method (see USP No. 5,536,637) is as follows.

Yeast such as Saccharomyces cerevisiae should secrete invertase into the medium in order to take sucrose or raffinose as a source of energy or

carbon. (Invertase is an enzyme to cleave raffinose into sucrose and melibiose, sucrose into fructose and glucose.) It is known that many known mammalian signal peptide make yeast secrete its invertase. From these knowledge, SST method was developed as a screening method to find novel signal peptide which make it possible can to secrete yeast invertase from mammalian cDNA library. SST method uses yeast growth on raffinose medium as a marker. Non-secretory type invertase gene SUC2 (GENBANK Accession No. V01311) lacking initiation codon ATG was inserted to yeast expression vector to prepare yeast SST vector pSUC2.

In this expression vector, ADH promoter, ADH terminator (both were derived from AAH5 plasmid (Gammerer, Methods in Enzymol. 101, 192-201, 1983)), 2u ori (as a yeast replication origin), TRP1 (as a yeast selective marker), ColE1 ori(as a E. Coli replication origin) and ampicillin resistance gene (as a drug resistance marker) were inserted. Mammalian cDNA was inserted into the upstream of SUC2 gene to prepare yeast SST cDNA library. Yeast lacking secretory type invertase, was transformed with this library. If inserted mammalian cDNA encodes a signal peptide, yeast could be survive in raffinose medium as a result of restoring secretion of invertase. Only to culture yeast colonies, prepare plasmids and determine the nucleotide sequence of the insert cDNAs, it is possible to identify novel signal peptide rapidly and easily.

Preparation of yeast SST cDNA library is as follows:

- (1) mRNA is isolated from the targeted cells, second-strand synthesis is performed by using random primer with certain restriction enzyme (enzyme I) recognition site,
- (2) double-strand cDNA is ligated to adapter containing certain restriction endonuclease (enzyme II) recognition site, differ from enzyme I, digested with enzyme I and fractionated in a appropriate size,

(3) obtained cDNA fragment is inserted into yeast expression vector on the upstream region of invertase gene which signal peptide is deleted and the library was transformed.

Detailed description of each step is as follows:

(1) mRNA is isolated from mammalian organs and cell lines stimulate them with appropriate stimulator if necessary) by known methods (Molecular Cloning (Sambrook, J., Fritsch, E. F. and Maniatis, T., Cold Spring Harbor Laboratory Press, 1989) or Current Protocol in Molecular Biology (F. M. Ausubel et al, John Wiley & Sons, Inc.) if not remark especially).

Mouse embryonic heart is chosen as a tissue source. Double-strand cDNA synthesis using random primer is performed by known methods.

Any sites may be used as restriction endonuclease recognition site I which is linked to adapter and restriction endonuclease recognition site II which is used in step (2), if both sites are different each other. Preferably, XhoI is used as enzyme I and EcoRI as enzyme II.

In step (2), cDNA is created blunt-ends with T4 DNA polymerase, ligated enzyme II adapter and digested with enzyme I. Fragment cDNA is analyzed with agarose-gel electrophoresis and is selected cDNA fraction ranging in size from 300 to 800 bp. As mentioned above, any enzyme may be used as enzyme II if it is not same the enzyme I.

In step (3), cDNA fragment obtained in step (2) is inserted into yeast expression vector on the upstream region of invertase gene which signal peptide is deleted. E. coli transformed with the expression vector. Many vectors are known as yeast expression plasmid vector. For example, YEp24 is also functioned in E. Coli. Preferably pSUC2 as described above is used.

Many host E. Coli strains are known for transformation, preferably DH10B competent cell is used. Any known transformation method is available, preferably it is performed by electropolation method. Transformant is

cultured by known methods to obtain cDNA library for yeast SST method.

However not every All of the clones do not contain cDNA fragment. Further all of the gene fragments do not encode unknown signal peptides. It is therefore necessary to screen a gene fragment encoding for an unknown signal peptide from the library.

Therefore, screening of fragments containing a sequence encoding an appropriate signal peptide is performed by transformation of the cDNA library into Saccharomyces cerevisiae (e.g. YT455 strain) which lack invertase (it may be prepared by known methods.). Transformation of yeast is performed by known methods, e.g. lithium acetate method. Transformant is cultured in a selective medium, then transferred to a medium containing raffinose as a carbon source. Survival colonies are selected and then prepared plasmid. Survival colonies on a raffinose-medium indicates that some signal peptide of secretory protein was inserted to this clone.

Isolated positive clones is determined the nucleotide sequence. As to a cDNA encodes unknown protein, full-length clone may be isolated by using cDNA fragment as a probe and then determined to obtain full-length nucleotide sequence. These manipulation is performed by known methods.

Once the nucleotide sequences shown in SEQ ID NO. 2, 5, 7, 10, 12 or 15 are determined partially or preferably fully, it is possible to obtain cDNA encode mammalian protein itself, homologue or subset of the invention.

cDNA library or mRNA derived from mammals was screened by PCR with any synthesized oligonucleotide primers or by hybridization with any fragment as a probe. It is possible to obtain cDNA encodes other mammalian homologue protein from other mammalian cDNA or genome library.

If a cDNA obtained above contains a nucleotide sequence of cDNA fragment obtained by SST (or concensus sequence thereof), it will be thought that the cDNA encodes signal peptide. So it is clear that the cDNA will be

full-length or almost full. (All signal peptides exist at N-termini of a protein and are encoded at 5'-temini of open reading frame of cDNA.)

The confirmation may be carried out by Northern analysis with the said cDNA as a probe. It is thought that the cDNA is almost complete length, if length of the cDNA is almost the same length of the mRNA obtained in the hybridizing band.

The present invention supplies full-length protein and also its mature protein sequence. The full-length protein sequence deduced from nucleotide sequences shown in SEQ ID NO. 2, 7 or 12. Mature proteins are obtained by expressing full-length cDNAs shown in SEQ ID NO. 3, 8 or 13 in mammalian cells or other host cells. Mature protein sequences are deduced from their full-length amino acid sequences.

Once the nucleotide sequences shown in SEQ ID NOs. 2, 5, 7, 10, 12 or 15 are determined, cDNAs of the present invention are obtained by chemical synthesis, or by hybridization making use of nucleotide fragments which are chemically synthesized as a probe. Furthermore, cDNAs of the present invention are obtained in desired amount by transforming a vector that contains the cDNA into a proper host, and culturing the transformant.

The polypeptides of the present invention may be prepared by:

- (1) isolating and purifying from an organism or a cultured cell,
- (2) chemically synthesizing, or
- (3) using recombinant DNA technology, preferably, by the method described in (3) in industrial production.

Examples of expression system for (host-vectoer system) producing a polypeptide by using recombinant DNA technology are the expression systems of bacteria, yeast, insect cells and mammalian cells.

In the expression of the polypeptide, for example, in E. Coli, the expression vector is prepared by adding the initiation codon (ATG) to 5'

end of a DNA encoding mature peptide, connecting the DNA thus obtained to the downstream of a proper promoter (e.g., trp promoter, lac promoter, λ PL promoter, T7 promoter etc.), and then inserting it into a vector (e.g., pBR322, pUC18, pUC19 etc.) which functions in an E. coli strain.

Then, an E. coli strain (e.g., E. coli DH1 strain, E. coli JM109 strain, E. coli HB101 strain, etc.) which is transformed with the expression vector described above may be cultured in a appropriate medium to obtain the desired polypeptide. When a signal peptide of bacteria (e.g., signal peptide of pel B) is utilized, the desired polypeptide may be also released in periplasm. Furthermore, a fusion protein with other polypeptide may be also produced easily.

In the expression of the polypeptide, for example, in a mammalian cells, for example, the expression vector is prepared by inserting the DNA shown in SEQ ID NO. 2, 5, 7, 10, 12 or 15 into the downstream of a proper promoter (e.g., SV40 promoter, LTR promoter, metallothionein promoter etc.) in a proper vector (e.g., retrovirus vector, papilloma virus vector, vaccinia virus vector, SV40 vector, etc.) a proper mammalian cell (e.g., monkey COS-7 cell, Chinese hamster CHO cell, mouse L cell etc.) is transformed with the expression vector thus obtained, and then the transformant is cultured in a proper medium to get a desired polypeptide in the culture medium. Further, fusion protein may be produced by linking cDNA fragment encoding other polypeptide such as Fc portion of an antibody. The polypeptide thus obtained may be isolated and purified by conventional biochemical methods.

Industrial Utility

The polypeptides of the present invention and cDNA encoding them are expected to exhibit one or more of the uses or biological activities (including those associated with assays cited herein) identified below.

Uses or activities described for proteins of the present invention may be provided by administration or use of such proteins or by administration or use of cDNA encoding them (such as, for example, in gene therapies or vectors suitable for introduction of DNA).

We have been confirmed that the said polypeptide possess the suppressing activity on the differentiation of vascular smooth muscle cells. Accordingly, the polypeptides may be useful for treatment of diseases related to abnormal proliferation of smooth muscle cells, for example, arteriosclerotic coronary disease, neointimal formation which results in restenosis after percutaneous transluminal coronary angioplasty and myosarcoma.

But not limit the present invention, the present polypeptide may show the following activity:

<Cytokine activity and cell proliferation/differentiation activity>

The protein of the present invention may exhibit cytokine, cell proliferation (either inducing or inhibiting) or cell differentiation (either inducing or inhibiting) activity or may induce production of other cytokines in certain cell populations.

Many protein factors discovered to date, including all known cytokines, have exhibited activity in one or more factor dependent cell proliferation assays, and hence the assays serve as a convenient confirmation of cytokine activity.

The activity of a protein of the present invention is evidenced by any one of a number of routine factor dependent cell proliferation assays for cell lines.

<Immune stimulating/suppressing activity>

The protein of the present invention may also exhibit immune

stimulating or immune suppressing activity. The protein may be useful in the treatment of various immune deficiencies and disorders (including severe combined immunodeficiency (SCID)), e.g., in regulating (up or down) growth and proliferation of T and/or B lymphocytes, as well as effecting the cytolytic activity of NK cells and other cell populations.

These immune deficiencies may be genetic or be caused by viral (e.g. HIV) as well as bacterial or fungal infections, or may result from autoimmune disorders.

More specifically, infectious diseases causes by viral, bacterial, fungal or other infection may be treatable using the protein of the present invention, including infections by HIV, hepatitis viruses, herpes viruses, mycobacteria, leshmania, malaria and various fungal infections such as candida. Of course, in this regard, a protein of the present invention may also be useful where a boost to the immune system generally would be indicated, i.e., in the treatment of cancer.

Such a protein of the present invention may also to be useful in the treatment of allergic reactions and conditions, such as asthma or other respiratory problems.

The protein of the present invention may also suppress chronic or acute inflammation, such as, for example, that associated with infection (such as septic shock or systemic inflammatory response syndrome (SIRS)), inflammatory bowel disease, Crohn's disease or resulting from over production of cytokines such as TNF or IL-I (such as the effect demonstrated by IL-11).

<Hematopoiesis regulating activity>

The protein of the present invention may be useful in regulation of hematopoiesis and, consequently, in the treatment of myeloid or lymphoid cell deficiencies. Even marginal biological activity in support of colony

forming cells or of factor-dependent cell lines indicates involvement in regulating hematopoiesis.

The said biological activities are concerned with the following all or some example(s). e.g. in supporting the growth and proliferation of erythroid progenitor cells alone or in combination with other cytokines, thereby indicating utility.

for example, in treating various anemias or for use in conjunction with irradiation/chemotherapy to stimulate the production of erythroid precursors and/or erythroid cells;

in supporting the growth and proliferation of myeloid cells such as granulocytes and monocytes/macrophages (i.e., traditional CSF activity) useful, for example, in conjunction with chemotherapy to prevent or treat consequent myelo-suppression;

in supporting the growth and proliferation of megakaryocytes and consequently of platelets thereby allowing prevention or treatment of various platelet disorders such as thrombocytopenia, and generally for use in place of or complimentary to platelet transfusions;

and/or in supporting the growth and proliferation of hematopoietic stem cells which are capable of maturing to any and all of the above-mentioned hematopoietic cells and therefore find therapeutic utility in various stem cell disorders (such as those usually treated with transplantation, including, without limitation, aplastic anemia and paroxysmal nocturnal hemoglobinuria), as well as in repopulating the stem cell compartment post irradiation/chemotherapy, either in-vivo or ex-vivo (i.e. in conjunction with bone marrow transplantation) as normal cells or genetically manipulated for gene therapy.

Suitable assays for proliferation and differentiation of various hematopoietic lines are cited above.

The activity of a protein of the invention may, among other means.

be measured by the following methods:

<Tissue generation/regeneration activity>

The protein of the present invention also may have utility in compositions used for bone, cartilage, tendon, Ligament and/or nerve tissue growth or regeneration, as well as for wound healing and tissue repair, and in the treatment of bums, incisions and ulcers.

The protein of the present invention, which induces cartilage and/or bone growth in circumstances where bone is not normally formed, has application in the healing of bone fractures and cartilage damage or defects in humans and other animals. Such a preparation employing the protein of the invention may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. De novo bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery.

The protein of this invention may also be used in the treatment of periodontal disease, and in other tooth repair processes. Such agents may provide an environment to attract bone-forming cells, stimulate growth of bone-forming cells or induce differentiation of progenitors of bone-forming cells. The protein of the invention may also be useful in the treatment of osteoporosis or osteoarthritis, such as through stimulation of bone and/or cartilage repair or by blocking inflammation or processes of tissue destruction (collagenase activity, osteoclast activity, etc.) mediated by inflammatory processes.

Another category of tissue regeneration activity that may be attributable to the protein of the present invention is tendon/ligament formation. A protein of the present invention, which induces

tendon/ligament-like tissue or other tissue formation in circumstances where such tissue is not normally formed, has application in the healing of tendon or ligament tears, deformities and other tendon or ligament defects in humans and other animals. Such a preparation employing a tendon/Ligament-like tissue inducing protein may have prophylactic use in preventing damage to tendon or ligament tissue, as well as use in the improved fixation of tendon or ligament to bone or other tissues, and in repairing defects to tendon or ligament tissue. De novo tendon/ligament-like tissue formation induced by a composition of the present invention contributes to the repair of congenital, trauma induced, or other tendon or ligament defects of other origin, and is also useful in cosmetic plastic surgery for attachment or repair of tendons or ligaments.

The compositions of the present invention may provide an environment to attract tendon- or ligament-forming cells, stimulate growth of tendon- or ligament-forming cells, induce differentiation of progenitors of tendon- or ligament-forming cells, or induce growth of tendon Ligament cells or progenitors ex vivo for return in vivo to effect tissue repair.

The compositions of the invention may also be useful in the treatment of tendinitis, carpal tunnel syndrome and other tendon or ligament defects. The compositions may also include an appropriate matrix and/or sequestering agent as a carrier as is well known in the art.

The protein of the present invention may also be useful for proliferation of neural cells and for regeneration of nerve and brain tissue. i.e. for the treatment of central and peripheral nervous system diseases and neuropathies. as well as mechanical and traumatic disorders, which involve degeneration, death or trauma to neural cells or nerve tissue.

More specifically, a protein may be used in the treatment of diseases of the peripheral nervous system, such as peripheral nerve injuries,

peripheral neuropathy and localized neuropathies, and central nervous system diseases, such as Alzheimer's, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome.

Further conditions which may be treated in accordance with the present invention include mechanical and traumatic disorders, such as spinal cord disorders, head trauma and cerebrovascular diseases such as stroke. Peripheral neuropathies resulting from chemotherapy or other medical therapies may also be treatable using a protein of the invention.

It is expected that the protein of the present invention may also exhibit activity for generation of other tissues, such as organs (including, for example, pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac) and vascular (including vascular endothelium) tissue, or for promoting or supressing the proliferation of cells comprising such tissues. Part of the desired effects may be by inhibition of fibrotic scarring to allow normal tissue to regenerate.

A protein of the present invention may also be useful for gut protection or regeneration and treatment of lung or liver fibrosis, reperfusion injury in various tissues, and conditions resulting from systemic cytokine damage. <Activin/Inhibin activity>

The protein of the present invention may also exhibit activin- or inhibin-related activities. Inhibins are characterized by their ability to inhibit the release of follicle stimulating hormone (FSH), while activins and are characterized by their ability to stimulate the release of follicle stimulating hormone (FSH).

Thus, a protein of the present invention. alone or in heterodimers with a member of the inhibin *a family, may be useful as a contraceptive based on the ability of inhibins to decrease fertility in female mammals and decrease spermatogenesis in male mammals. Administration of sufficient

amounts of other inhibins can induce infertility in these mammals.

Alternatively, the protein of the invention, as a homodimer or as a heterodimer with other protein subunits of the inhibin-*b group, may be useful as a fertility inducing therapeutic, based upon the ability of activin molecules in stimulating FSH release from cells of the anterior pituitary. See for example, USP 4,798,885. The polypeptide of the invention may also be useful for advancement of the onset of fertility in sexually immature mammals, so as to increase the lifetime reproductive performance of domestic animals such as cows, sheep and pigs.

<Chemotactic/chemokinetic activity>

A protein of the present invention may have chemotactic or chemokinetic activity (e.g., act as a chemokine) for mammalian cells, including. for example. monocytes, neutrophils, T-cells, mast cells, eosinophils and/or endothelial cells.

Chemotactic and chemokinetic proteins can be used to mobilized or attract a desired cell 'population to a desired site of action. Chemotactic or chemokinetic proteins provide particular advantages in treatment of wounds and other trauma to tissues, as well as in treatment of localized infections. For example, attraction of lymphocytes, monocytes or neutrophils to tumors or sites of infection may result in improved immune responses against the tumor or infecting agent.

A protein or peptide has chemotactic activity for a particular cell population if it can stimulate, directly or indirectly, the directed orientation or movement of such cell population. Preferably, the protein or peptide has the ability to directly stimulate directed movement of cells. Whether a particular protein has chemotactic activity for a population of cells can be readily determined by employing such protein or peptide in any known assay for cell chemotaxis.

<Hemostatic and thrombolytic activity>

The protein of the invention may also exhibit hemostatic or thrombolyic activity. As a result, such a protein is expected to be useful in treatment of various coagulation disorders (including hereditary disorders, such as hemophilias) or to enhance coagulation and other hemostatic events in treating wounds resulting from trauma, surgery or other causes. A protein of the invention may also be useful for dissolving or inhibiting formation of thromboses and for treatment and prevention of conditions resulting therefrom (such as. for example, infarction or stroke).

<Receptor/ligand activity>

The protein of the present invention may also demonstrate activity as receptors, receptor ligands or inhibitors or agonists of receptor/ligand interactions. Examples of such receptors and ligands include, without limitation, cytokine receptors and their ligands, receptor kinases and their ligands, receptor phosphatases and their ligands, receptors involved in cell-cell interactions and their ligands (including without limitation. cellular adhesion molecules (such as selectins, integrins and their ligands) and receptor/ligand pairs involved in antigen presentation, antigen recognition and development of cellular and humoral immune responses).

Receptors and ligands are also useful for screening of potential peptide or small molecule inhibitors of the relevant receptor/ligand interaction. A protein of the present invention (including, without limitation, fragments of receptors and ligands) may themselves be useful as inhibitors of receptor/ligand interactions.

<Nutritional uses>

Proteins of the present invention can also be sued as nutritional sources or supplements. Such uses include without limitation use as a protein or amino acid supplement, use as a carbon source, use as a nitrogen source

and use as a source of carbohydrate. In such cases the protein of the present invention can be added to the feed of a particular organism or can be administered as a separate solid or liquid preparation, such as in the form of powder, pills, solutions, suspensions or capsules. In the case of microorganisms, the protein of the invention can be added to the medium in or on which the microorganism is cultured.

<Cadherin/Tumor invasion suppresser activity>

Cadherins are calcium-dependent adhesion molecules that appear to play major roles during development, particularly in defining specific cell types. Loss or alteration of normal cadherin expression can lead to changes in cell adhesion properties linked to tumor growth and metastasis. Cadherin malfunction is also implicated in other human diseases, such as pemphigus vulgaris and pemphigus foliaceus (autoimmune blistering skin diseases), Crohn's disease, and some developmental abnormalities.

The cadherin superfamily includes well over forty members, each with a distinct pattern of expression. All members of the superfamily have in common conserved extracellular repeats (cadherin domains), but structural differences are found in other parts of the molecule. The cadherin domains bind calcium to form their tertiary structure and thus calcium is required to mediate their adhesion. Only a few amino acids in the first cadherin domain provide the basis for homophilic adhesion;

modification of this recognition site can change the specificity of a cadherin so that instead of recognizing only itself, the mutant molecule can now also bind to a different cadherin. In addition, some cadherins engage in heterophilic adhesion with other cadherin.

E-cadherin, one member of the cadherin superfamily, is expressed in epithelial cell types. Pathologically, if E-cadherin expression is lost in a tumor, the malignant cells become invasive and the cancer metastasizes.

Transfection of cancer cell line with cDNAs expressing E-cadherin has reversed cancer-associated changes by returning altered cell shapes to normal, restoring cells adhesiveness to each other and to their substrate, decreasing the cell growth rate, and drastically reducing anchorage-independent cell growth.

Thus, reintroducing E-cadherin expression reverts carcinomas to a less advanced stage. It is likely that other cadherins have the same invasion suppresser role in carcinomas derived from other tissue types. Therefore, proteins of the present invention with cadherin activity, and cDNAs of the present invention encoding such proteins, can be used to treat cancer. Introducing such proteins or cDNAs into cancer cells can reduce or eliminate the cancerous change observed in these cells by providing normal cadherin expression.

Cancer cells have also been shown to express cadherins of a different tissue type than their origin, thus allowing these cells to invade and metastasize in a different tissue in the body. Proteins of the present invention with cadherin activity, and cDNAs of the present invention encoding such proteins, can be substituted in these cells for the inappropriately expressed cadherins, restoring normal cell adhesive properties and reducing or eliminating the tendency of the cells to metastasize.

Additionally, proteins of the present invention with cadherin activity, and cDNA of the present invention encoding such proteins, can used to generate antibodies recognizing and binding to cadherins. Such antibodies can be used to block the adhesion of inappropriately expressed tumor-cell cadherins, preventing the cells from forming a tumor elsewhere. Such an anti-cadherin antibody can also be used as a marker for the grade, pathological type, and prognosis of a cancer, i.e. the more progressed the cancer, the less cadherin expression there will be, and this decrease in cadherin expression can be

detected by the use of a cadherin-binding antibody.

Fragments of proteins of the present invention with cadherin activity, preferably a polypeptide comprising a decapeptide of the cadherin recognition site, and cDNAs of the present invention encoding such protein fragments, can also be used to block cadherin function by binding to cadherins and preventing them from binding in ways that produce undesirable effects.

Additionally, fragments of proteins of the present invention with cadherin activity, preferably truncated soluble cadherin fragments which have been found to be stable in the circulation of cancer patients, and polynulceotides encoding such protein fragments, can be used to disturb proper cell-cell adhesion.

<Tumor Inhibiting activity>

In addition to the activities described above for immunological treatment or prevention of tumors, the protein of the invention may exhibit other anti-tumor activities. The protein may inhibit tumor growth directly or indirectly (such as, for example, via ADCC). The protein may exhibit its tumor inhibitory activity by acting on tumor tissue or tumor precursor tissue, by inhibiting formation of tissues necessary to support tumor growth (such as, for example, by inhibiting angiogenesis), by causing production of other factors, agents or cell types which inhibit tumor growth, or by suppressing, eliminating or inhibiting factors, agents or cell types which promote tumor growth.

<Other activity>

The protein of the invention may also exhibit one or more of the following additional activities or effects: inhibiting the growth, infection or function of, or killing, infectious agents, including, bacteria, viruses, fungi and other parasites;

effecting (suppressing or enhancing) bodily characteristics, including,

height, weight, hair color, eye color, skin, fat to lean ratio or other tissue pigmentation, or organ or body part size or shape (such as, for example, breast augmentation or diminution);

effecting elimination of dietary fat, protein, carbohydrate;
effecting behavioral characteristics, including appetite, libido, stress,
cognition (including cognitive disorders), depression and violent
behaviors;

providing analgesic effects or other pain reducing effects; promoting differentiation and growth of embryonic stem cells in lineages other than hematopoietic lineages;

in the case of enzymes, correcting deficiencies of the enzyme and treating deficiency-related diseases.

The polypeptide with above activities, is suspected to have following functions by itself or interaction with its ligands or receptors or association with other molecules. For example, proliferation or cell death of B cells, T cells and/or mast cells or class specific induction of B cells by promotion of class switch of immunoglobulin genes; differentiation of B cells to antibody-forming cells; proliferation, differentiation, or cell death of precursors of granulocytes; proliferation, differentiation, or cell death of precursors of monocytes-macrophages;

proliferation, of up regulation or cell death of neutrophils, monocytes-macrophages, eosinophils and/or basophils; proliferation, or cell death of precursors of megakaryocytes; proliferation, differentiation, or cell death of precursors of neutrophils; proliferation, differentiation, or cell death of precursors of T cells and B cells; promotion of production of erythrocytes; sustainment of proliferation of erythrocytes, neutrophils, eosinophils, basophils, monocytes-macrophages, mast cells, precursors of megakaryocyte; promotion of migration of neutrophils, monocytes-

macrophages, B cells and/or T cells; proliferation or cell death of thymocytes; suppression of differentiation of adipocytes; proliferation or cell death of natural killer cells;

proliferation or cell death of hematopoietic stem cells; suppression of proliferation of stem cells and each hematopoietic precursor cells; promotion of differentiation from mesenchymal stem cells to osteoblasts or chondrocytes, proliferation or cell death of mesenchymal stem cells, osteoblasts or chondrocytes and promotion of bone absorption by activation of osteoclasts and promotion of differentiation from monocytes to osteoclasts.

This peptide is also suspected to function to nervous system, so expected to have functions below; differentiation to kinds of neurotransmitter-responsive neurons, survival or cell death of these cells; promotion of proliferation or cell death of glial cells; spread of neural dendrites; survival or cell death of gangriocytes; proliferation, promotion of differentiation, or cell death of astrocytes; proliferation or survival of peripheral neurons; proliferation or cell death of Schwann cells; proliferation, survival or cell death of motoneurons.

Furthermore, in the process of development of early embryonic, this polypeptide is expected to promote or inhibit the organogenesis of epidermis, brain, backbone, and nervous system by induction of ectoderm, that of notochord connective tissues(bone, muscle, tendon), hemocytes, heart, kidney, and genital organs by induction of mesoderm, and that of digestive apparatus (stomach, intestine, liver, pancreas), respiratory apparatus (lung, trachea) by induction of endoderm. In adult, also, this polypeptide is thought to proliferate or inhibit the above organs.

Therefore, this polypeptide itself is expected to be used as an agent for the prevention or treatment of disease of progression or suppression of immune, nervous, or bone metabolic function, hypoplasia or overgrowth

of hematopoietic cells: inflammatory disease (rheumatism, ulcerative colitis, etc.), decrease of hematopoietic stem cells after bone marrow transplantation, decrease of leukocytes, platelets, B-cells, or T-cells after radiation exposure or chemotherapeutic dosage against cancer or leukemia, anemia, infectious disease, cancer, leukemia, AIDS, bone metabolic disease(osteoporosis etc.), arteriosclerosis, various degenerative disease (Alzheimer's disease, multiple sclerosis, etc.), or nervous lesion.

In addition, since this polypeptide is thought to induce the differentiation or growth of organs derived from ectoderm, mesoderm, and endoderm, this polypeptide is expected to be an agent for tissue repair (epidermis, bone, muscle, tendon, heart, kidney, stomach, intestine, liver, pancreas, lung, and trachea, etc.).

Quantitation of this polypeptide in the body can be performed using polyclonal or monoclonal antibodies against this polypeptide. It can be used the study of relationship between this polypeptide and disease or diagnosis of disease, and so on. Polyclonal and monoclonal antibodies can be prepared using this polypeptide or its fragment as an antigen by known method.

Identification, purification or molecular cloning of known or unknown proteins which bind this polypeptide can be performed using this polypeptide by, for example, preparation of the affinity-column.

Identification of the molecules which interact with this polypeptide and molecular cloning of the gene can be performed by west-western method using this polypeptide or by yeast two-hybrid system using the cDNA (preferably cDNA encoding this polypeptide).

Agonists/antagonists of this receptor polypeptide and inhibitors between receptor and signal transduction molecules can be screened using this polypeptide.

For example, the screening can be carried out the following method.

- a) The reaction mixtures, which contain this polypeptide, screening compound and the cells, are incubated under the condition which the cells are normally stimulated by this peptide. (The reaction mixtures also contain the labeled compound, which is introduced into the cells according to the cell proliferation, and peptide which allow to observe the function of this peptide efficiently.)
- b) Decision that the compounds are efficient agonists/antagonists or not, are performed by measurement of cell proliferation ability.

More detailed methods are followed:

Rat vascular muscle cell line (ATCC CRL-1444 or CRL1476) is cultured in 96 well plate with 10%FBS for 24 hours. Then the culture medium are replaced to the serum-free medium supplemented with each several concentrations of human PDGF-BB. At that time compounds to screen as well as A55 protein are added in the medium when screening the antagonists of A55 protein. While, compounds alone are added in the medium when screening the agonists of A55 protein. After 24 hours incubation, these cells are pulsed for 4hours with 3H-thymidine. By measuring the 3H-thymidine incorporation, it is possible to determine whether the compounds have inhibitory or stimulatory effect on the A55 activity.

cDNAs of the present invention are useful not only the important and essential template for the production of the polypeptide of the present invention which is expected to be largely useful, but also be useful for diagnosis or therapy (for example, treatment of gene lacking, treatment to stop the expression of the polypeptide by antisense DNA (RNA)).

Genomic DNA may be isolated with the cDNA of the present invention, as a probe. As the same manner, a mouse or human gene encoding which can be highly homologous to the cDNA of the present invention, that is, which encodes a polypeptide highly homologous to the polypeptide of the present

invention and a gene of animals excluding mouse or human which can be highly homologous to the cDNA of the present invention, also may be isolated.

Application for Pharmaceuticals

For the medical treatment for diseases described above, the polypeptide of the invention or the antibody of the polypeptide of the invention may be administered systemically or partially in most cases, usually by oral or parenteral administration, preferably orally, intravenously or intraventricularly.

The doses to be administered depend upon age, body weight, symptom, desired therapeutic effect, route of administration, and duration of the treatment etc. In human adults, one dose per person is generally between 100 µg and 100 mg, by oral administration, up to several times per day, and between 10 µg and 100 mg, by parenteral administration up to several times per day.

As mentioned above, the doses to be used depend upon various conditions. Therefore, there are cases in which doses lower than or greater than the ranges specified above may be used.

The compounds of the present invention, may be administered as solid compositions, liquid compositions or other compositions for oral administration, as injections, liniments or suppositories etc. for parenteral administration.

Solid compositions for oral administration include compressed tablets, pills, capsules, dispersible powders, granules. Capsules include soft or hard capsules.

In such compositions, one or more of the active compound(s) is or are admixed with at least one inert diluent (such as lactose, mannitol, glucose, hydroxypropyl cellulose, microcrystalline cellulose, starch,

polyvinylpyrrolidone, magnesium metasilicate aluminate, etc.). The compositions may also comprise, as is normal practice, additional substances other than inert diluents: e.g. lubricating agents (such as magnesium stearate etc.), disintegrating agents (such as cellulose calcium glycolate, etc.), stabilizing agents (such as human serum albumin, lactose etc.), and assisting agents for dissolving (such as arginine, asparaginic acid etc.).

The tablets or pills may, if desired, be coated with a film of gastric or enteric materials (such as sugar, gelatin, hydroxypropyl cellulose or hydroxypropylmethyl cellulose phthalate, etc.), or be coated with more than two films. And then, coating may include containment within capsules of absorbable materials such as gelatin.

Liquid compositions for oral administration include pharmaceutically-acceptable emulsions, solutions, syrups and elixirs. In such compositions, one or more of the active compound(s) is or are contained in inert diluent(s) commonly used (purified water, ethanol etc.). Besides inert diluents, such compositions may also comprise adjuvants (such as wetting agents, suspending agents, etc.), sweetening agents, flavoring agents, perfuming agents, and preserving agents.

Other compositions for oral administration include spray compositions which may be prepared by known methods and which comprise one or more of the active compound(s). Spray compositions may comprise additional substances other than inert diluents: e.g. stabilizing agents (sodium sulfite etc.), isotonic buffer (sodium chloride, sodium citrate, citric acid, etc.). For preparation of such spray compositions, for example, the method described in the United States Patent No. 2,868,691 or 3,095,355 (herein incorporated in their entireties by reference) may be used.

Injections for parenteral administration include sterile aqueous or

non-aqueous solutions, suspensions and emulsions. In such compositions, one or more active compound(s) is or are admixed with at least one inert aqueous diluent(s) (distilled water for injection, physiological salt solution, etc.) or inert non-aqueous diluents(s)(propylene glycol, polyethylene glycol, olive oil, ethanol, POLYSOLBATE 80 TM, etc.).

Injections may comprise additional compound other than inert diluents: e.g. preserving agents, wetting agents, emulsifying agents, dispersing agents, stabilizing agent (such as human serum albumin, lactose, etc.), and assisting agents such as assisting agents for dissolving (arginine, asparaginic acid, etc.).

The Best Mode of the Invention

The following examples concerning clone A55 are illustrated, but not limit the present invention.

Example 1

Preparation of poly(A)+RNA

Total RNA was prepared from mouse day18.5 embryonic heart by TRIzol reagent (Trade Mark, GIBCOBRL), and poly (A) + RNA was purified from the total RNA by mRNA Purification Kit (Trade Mark, Pharmacia).

Example 2

Preparation of yeast SST cDNA library

Double strand cDNA was synthesized by SuperScript Plasmid System for cDNA Synthesis and Plasmid Cloning (brand name, GIBCOBRL) with above poly(A)+RNA as template and random 9mer as primer which was containing XhoI site:

5'-CGA TTG AAT TCT AGA CCT GCC TCG AGN NNN NNN NN-3' (SEQ ID NO. 16)

cDNA was ligated EcoRI adapter by DNA ligation kit ver.2 (trade name, Takara Shuzo; this kit was used in all ligating steps hereafter.) and digested by XhoI. cDNAs were separated by agarose-gel electrophoresis. 300 - 800 bp cDNAs were isolated and were ligated to EcoRI/NotI site of pSUC2 (see US 5,536,637). E. Coli DH10B strain were transformed by pSUC2 with electropolation to obtain yeast SST cDNA library.

Example 3

Screening by SST method and DNA sequencing of positive clone

Plasmids of the cDNA library were prepared. Yeast YTK12 strain were transformed by the plasmids with lithium acetate method (Current Protocols In Molecular Biology 13.7.1). The transformed yeast were plated on triptphan-free medium (CMD-Try medium) for selection. The plate was incubated for 48 hour at 30 oc. Replica of the colony which is obtained by Accutran Replica Plater (trade name, Schleicher & Schuell) were place YPR plate containing raffinose for carbon source, and the plate was incubated for 14 days at 30 oc.

After 3 days, each colony appeared was streaked on YPR plate again. The plates were incubated for 48 hours at 30 oC. Single colony was inoculated to YPR medium and was incubated for 48 hours at 30 oC. Then plasmids were prepared. Insert cDNA was amplified by PCR with two kind primers which exist end side of cloning site on pSUC2 (sense strand primers were biotinylated). Biotinylated single strand of cDNAs were purified with Dynabeads (trade name, DYNAL) and determined the nucleotide sequences.

Sequencing was performed by Dye Terminator Cycle Sequencing Ready Reaction with DNA Sequencing kit (trade name, Applied Biosystems Inc.) and sequence was determined by DNA sequencer 373 (Applied Biosystems Inc.). All

sequencing hereafter was carried with this method.

The clone named A55 is not registered on databases by homology search of cDNA sequence and deduced amino acid sequence and so it is cleared that the sequence is novel one. So, we tried to isolate clone full-length cDNA of the fragment of A55 clone (hereafter A55 SST fragment cDNA). We confirmed that A55 SST fragment cDNA contains signal peptide by comparison with known peptide which has signal peptide in view of function and structure.

Example 4

Cloning and sequencing of a full-length cDNA of A55

Phage particles of a cDNA library of mouse day13 embryonic heart(uni-ZAP XR, Stratagene) were transfected to E. coli XL1-Blue MRF* host cells (Stratagene). Obtained one million plaques were transferred to nylon membranes. The membranes were hybridized with 32P-labeled mouse A55 SST fragment cDNA as a probe. Many positive plaques were obtained.

From one positive plaque the phage particles containing a cloned insert were prepared, and were subjected to conversion into phagemid particles (pBluescript SK(-)) by co-infection of E. coli XL1-Blue MRF* host cells(Stratagene) with ExAssist helper phage(Stratagene). The phagemid particles were transfected to E. coli DH5a. The plasmids were prepared from the obtained transformants.

Nucleotide sequence of 5'-end of cDNA were determined to confirm the existence of the sequences of SST fragment cDNA. And then full-length sequencing were performed to obtain SEQ ID NO.3.

An open reading frame was determined and translation region for amino acid sequence shown in SEQ ID NO. 2 and deduced full-length amino acid sequence shown in SEQ ID NO. 1 were obtained. Mature protein of the said polypeptide was deduced to 425 amino acids shown in SEQ ID NO. 3 (144..1418) or 423 amino

acids shown in SEQ ID NO. 4. Translation region of SEQ ID NO. 4 is shown in SEQ ID NO. 5.

It was confirmed that there was no identical sequences to the DNA of the present invention by homology search program, BLASTN and FASTA against public nucleotide database. And it was also confirmed that there were no identical sequences to the polypeptide of the present invention (mouse A55 protein) by homologue search program, BLASTX, BLASTP and FASTA against amino acid database.

It is revealed that the polypeptide of the present invention, mouse A55 is novel secretion protein since the polypeptide have no trans-membrane region by hydrophobisity analysis of the amino acid sequence.

It was revealed that A55 protein contained six EGF like domains by motif search, so it was expected that clone A55 also possesses EGF family like activities. Significant homology were also recognized between the amino acid sequence of clone mouse A55 (1-448 AA region) and the one of human S1-5 (SwissProt Accession No. HSU03877) (1-387 AA region) by the comparison using BLASTX, BLASTP and FASTA. It was reported that human S1-5 was a secreted protein containing EGF like domain, was induced in fibroblasts by growth arrest, and stimulated DNA synthesis (Beata Lecka-Czernik et. al. Mol. Cell. Biol. 15, 120-128, 1995). Farther it was revealed that A55 protein was homologous to many proteins containing EGF-like domain.

Example 5

Isolation of isoform gene of mouse A55 protein

Initiation coden was determined by cloning of 5'-end cDNA by 5'-RACE (Rapid Amplification of cDNA Ends method using Marathon cDNA Amplification Kit (trade name, Clontech). Double stranded cDNA template was prepared from poly(A)+RNA of mouse embryonic heart tissue. Primer mA55-R1:

5'- CGT TTG TGC ACT GCT GCT GTG CAT TCC -3' (SEQ ID NO. 17)

was prepared based on the information of full-length nucleotide sequences.

PCR was performed with the said primer and adapter primer attached in the kit.

Amplified cDNA was separated with agarose-gel electrophoresis, and to pGEM-T Vector (trade name, Promega), ligated in and transformed to E. Coli DH5a and then plasmid was prepared. The full-length nucleotide sequences were determined. We found two deferent 5'-end sequences. One was identical to the clone containing the sequence in SEQ ID NO. 3, the other contained unknown sequence and no translational start site ATG (See SEQ ID NO. 7 and 8).

The region defined from exon 1 of the clone was replaced by another exon which exists 400 bp downstream region of exon 1 was clarified by gene analysis. So it was cleared that the clone shown in SEQ ID NO. 8 was generated by alternative splicing of exon 1. The clone encodes isoform protein shown in SEQ ID NO. 6 (6 amino acids in N termini of SEQ ID NO.1 was replaced by 19 amino acids in N termini of SEQ ID NO. 6).

The mature protein of this polypeptide was deduced 425 amino acids shown in SEQ ID NO. 8 (340...1614) or 423 amino acids shown in SEQ ID NO. 9. SEQ ID NO. 10 is the translational region of the polypeptide shown in SEO ID NO. 9.

Example 6

Determination of nucleotide sequence of human A55 gene

The present inventors found that Human EST sequence (GENBANK Accession No. H17726) homologous to 5'-end sequence of mouse A55 in the process of

homology search shown in example 4.

And the present inventors buy the Clone ID 50483 derived from human brain cDNA library GENBANK Accession No. H17726 from American Type Culture Collection (ATCC). The full-length nucleotide sequence shown in SEQ ID No. 13 was determined with the same manner as in the determination of mouse A55. Open reading frame was determined and translational region shown in SEQ ID No. 12 and deduced amino acid sequence shown in SEQ ID No. 11 were obtained.

From above results, it is clarified that the human clone is full-length and have 89.3 % homology to mouse A55 at DNA level (translational region) and have 94.2 % homology to the one at amino acid level. It is suggested that the obtained human clone should be human counterpart of mouse A55. (The clone was called human A55 hereafter.)

The mature protein of this polypeptide was deduced 425 amino acids shown in SEQ ID NO. 13 (238...1512) or 423 amino acids shown in SEQ ID NO. 14. Translational region of the polypeptide shown in SEQ ID NO. 14 shows in SEQ ID NO. 15.

It was confirmed that there was no identical sequences to the DNAs of the present invention by homology search program, BLASTN and FASTA against public nucleotide database. And it was also confirmed that there were no identical sequences to human A55 proteins by homologue search program, BLASTX, BLASTP and FASTA against amino acid database.

Example 7

Mouse A55 protein expression in mammalian cell

Mouse full-length cDNA shown in SEQ ID NO. 3 was inserted into expression vector for mammalian cell pNotS (Kaufman et al., Nucleic Acids Res. 19, 4485-4490 (1991)) and mouse A55 expression plasmid pNotS-mA55 was constructed.

293T cells (which is derived from 293 cells (ATCC CRL-1573) and it stably transfected with SV40 T antigen) were transfected with pNotS and pNotS-mA55 using lipofection (GIBCOBRL). After preincubated for 19 hours, the cells were pulsed for 30 minutes with ³⁵S-Met in the Met-free medium. Then the cells were incubated in the medium containing Met for 5 hours. Supernatant of the cells was recovered and concentrated 10-fold using centricon-10 (trade name, AMICON). Samples were subjected to SDS-polyacrylamide-gel electrophoresis. The gel was dried and ³⁵S-labeled proteins were detected with BAS 2000 (Fuji Film).

A band was detected at 60-70 kDa in the supernatant of pNotS-mA55-transfected 293T cells. This band was not detected in the supernatant of pNotS-transfected 293T cells. This results confirmed that recombinant mouse A55 protein was expressed and secreted into the medium. Molecular weight (60-70 kDa) of this recombinant mouse A55 protein was greater than it (48 kDa) predicted from its amino acid sequences. As this protein had two potential N-linked glycosylation sites and many Ser and Thr residues in which O-linked glycosyl chain could be added , it was suggested that the mouse A55 protein was a glycoprotein.

Example 8

Measurement of inhibition on proliferation of rat vascular smooth muscle cells by mouse A55 protein

Vascular smooth muscle cells were isolated from rat aorta ranging from heart to diaphragm and cultured primarily by the methods described in Shin Seikagaku Jikken Kouza 10 (The Japanese Biochemical Society). These cells were co-incubated with 1, 3 or 10 ng/ml of human recombinant PDGF-BB (Genzyme) and 10% (v/v) of the mock or mA55 supernatant prepared according to the method described in example 7. And BrdU incorporation was measured using a Cell

Proliferation ELISA, BrdU colorimetric kit (Boehringer-Mannheim).

The supernatant from 293T cells transfected with pNotS-mA55 significantly inhibited BrdU incorporation of rat primary vascular smooth muscle cells, while the supernatant from 293T cells transfected with only pNotS show no effect as shown in Fig. 1.

Moreover the supernatant from 293T cells transfected with pNotS-mA55 also inhibited BrdU incorporation even when rat vascular smooth muscle cells were stimulated with 1, 3 or 10 ng/ml of PDGF and increased BrdU incorporation in a dose-dependent manner, whereas the supernatant from 293T cells transfected with only pNotS did not affect compared with no supernatant addition (See Fig. 1).

These data revealed that the recombinant mouse A55 protein had the growth inhibitory activity on vascular smooth muscle cells.

Example 9

Human A55 protein expression in mammalian cell

Human A55 expression plasmid, pNotS-hA55, was constructed by inserting human full-length cDNA shown in SEQ ID NO. 13 into was into expression vector for mammalian cell pNotS (Kaufman et al., Nucleic Acids Res. 19, 4485-4490 (1991)).

Cosl cells were transfected with pNotS and pNotS-hA55 by lipofectin (GIBCOBRL). After preincubation for 24 hours, the cells were pulsed for 5 hours with 35S-Met and 35S-Cys. Supernatant of the cells was recovered and concentrated to 10-fold using centricon-10 (trade name, AMICON). Proteins in concentrated supernatant were separated by electrophoresis through SDS-page. The gel was dried and 35S-labeled proteins were detected with BAS 2000 (Fuji Film).

A band was detected at 60-70 kDa in the supernatant from Cosl cells

transfected with pNotS-hA55. This band was not detected in the supernatant of pNotS transfected Cos1 cells. These results confirmed that recombinant human A55 protein was expressed and secreted into the medium. And human A55 protein was also suggested that sugar chains were also added to human A55 protein as well as mouse A55.

Example 10

Detection of the inhibitory activity on proliferation of rat vascular smooth muscle cells by human A55 protein

A DNA fragment encoding a signal sequence of honey bee mertin, a tag sequence of six His residues and an enterokinase cleavage site was added to 5'-end of human A55 cDNA sequence from 238 to 1515 in SEQ ID NO. 13 or sequence in SEQ ID NO. 15 followed by stop codon and inserted into expression vector pNots. Cos1 cells were transfected with pNotS-hA55 plasmid DNA and pNotS control plasmid DNA. The supernatant was recovered , digested by enterokinase, pulled through nickel column to remove the linker peptide, and then concentrated 10-fold by centricon-10 (trade name, AMICON).

After rat vascular muscle cell line (ATCC CRL-1444) was cultured in 96 well plates with 10% FBS for 24 hours, these cells were incubated for 24 hours with serum-free medium supplemented with several concentrations (1, 10 or 10 ng/ml) of human PDGF-BB (Genzyme) and with 10 % total volume of supernatant of Cos1 cells which were transfected with pNotS-hA55 or pNotS, and then were pulsed for 4 hours with 3H-thymidine. After harvesting 3H-thymidine incorporation was detected. In this cell line remarkable decrease of 3H-thymidine incorporation were observed by supplement with hA55 supernatant, while there was no effect in the presence of the control supernatant.

Moreover similar effects were also observed when using other rat

vascular smooth muscle cell lines (ATCC CRL-1476 and CRL-2018) and human vascular smooth muscle cell line (ATCC CRL-1999). These results revealed that the recombinant human A55 protein also had the growth inhibitory activity on the vascular smooth muscle cells as well as mouse A55 protein.

Morphological change was observed on the vascular smooth muscle cells treated with the supernatant from hA55-transfected Cos1 cells by microscopy detection. While no morphological change was observed on melanoma cell line SK-MEL-28 at the same experiment. Furthermore, hA55 protein was observed to induce the expression of chemokine JE and JK.

Experiment 11

Preparation of anti mouse A55 polyclonal antibody

Three kinds of peptide fragments of mouse A55 were synthesized by solid phase method:

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RTNPVYRGPYSNPYSTSYSG (71-90) (48-67 of SEQ ID NO. 1).

GAYYIFQIKSGNEGREFYMR (376-395) (353-372 of SEQ ID NO. 1).

MTRPIKGPRDIQLDLEMITVN (406-426) (383-403 of SEQ ID NO. 1).
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Rabbits were immunized to these peptides as immunogen and the serum were prepared after measurement of the activity. Each anti-mouse A55 antibody was purified by affinity column immobilized each peptide which was used as immunogen from the obtained serum.

The supernatant prepared by the same method described in example 7, was subjected to SDS-PAGE, the separated proteins were transferred to Immobilon-P (PVDF membrane, trade name, Millopore) from the acrylamide gel. After blocking the membranes they were incubated with the anti mouse A55 polyclonal antibody as the first antibody and by developing using ECL kit

(Amersham), the recombinant mouse A55 protein was detected.

A 60 k Da band was detected in the supernatant from mA55 transfected Cosl cells as well as 35S-labeling experiment described in example 7. While no bands were detected in the supernatant from mock-transfected Cosl cells. These results confirmed that the obtained polyclonal antibodies specifically recognized the mouse A55 protein.

Claims

- 1. Substantially purified form of the polypeptide that comprising the amino-acid sequence shown in SEQ ID NO. 11 or 14, homologue thereof, fragment thereof or homologue of the fragment.
- 2. A polypeptide according to claim 1 that consists (comprising) of the amino-acid sequence shown in SEQ ID NO. 11 or 14.
 - A cDNA encoding the polypeptide according to claim 1.
- 4. A cDNA according to claim 3 that comprising the nucleotide sequence shown in SEQ ID NO. 12 or 15 or a fragment cDNA selectively hybridized to the cDNA.
- 5. A cDNA according to claim 3 that comprising the nucleotide sequence shown in SEQ ID NO. 13 or a fragment cDNA selectively hybridized to the cDNA.
- 6. A replication or expression vector carrying the cDNA according to claim 3 to 5.
- 7. A host cell transformed with the replication or expression vector according to claim 6.
- 8. A method for producing the polypeptide according to claim 1 or 2 which comprises culturing a host cell according to claim 7 under a condition effective to express the polypeptide according to claim 1 or 2.
- 9. A monoclonal or polyclonal antibody against the polypeptide according to claim 1 or 2.
- 10. A pharmaceutical composition containing the polypeptide according to claim 1 or 2 or the antibody according to claim 9, in association with pharmaceutically acceptable diluent and/or carrier.
- 11. A pharmaceutical composition for the treatment of abnormal growth of smooth muscle cell, containing a polypeptide according to claim 1 or 2, in association with a pharmaceutically acceptable diluent and/or

carrier.

- 12. A pharmaceutical composition for the treatment of arteriosclerosis, restenosis after PTCA or myosarcoma, containing the polypeptide according to claim 1 or 2, in association with a pharmaceutically acceptable diluent and/or carrier.
- 13. A screening method for an antagonist or agonist of the polypeptide according to claim 1 or 2 with using the said polypeptide.

Abstract

A novel human polypeptide. Because of having an effect of inhibiting the proliferation of vascular smooth muscle cells, this polypeptid is applicable to the treatment of diseases in which abnormal smooth muscle proliferation participates, for example, arteriosclerosis and myeroma. Moreover, this polypeptide has hematopoietic cell regulatory activity, tissue forming/reparing activity, activin/inhibin activity, chemotactic/chemokinetic activity, blood coagulating and thrombotic activity, etc. Thus, it seems useful in preventing and/or treating various diseases.

Fig. 1

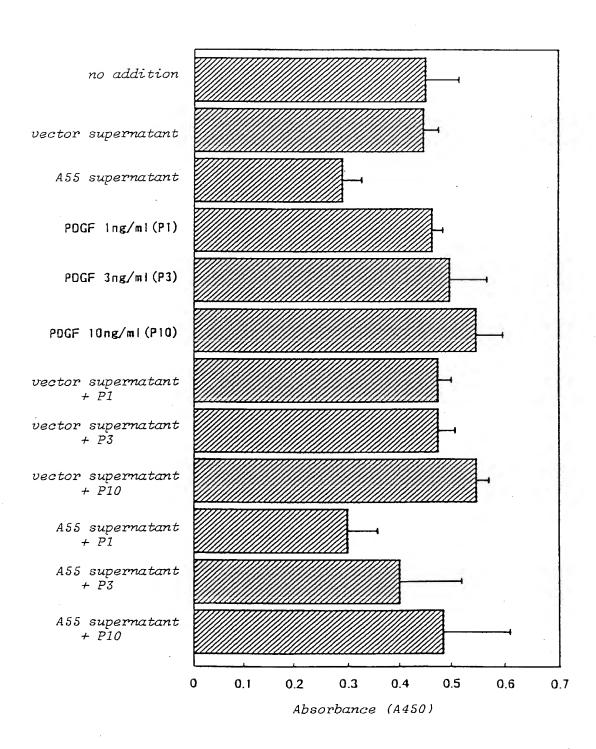
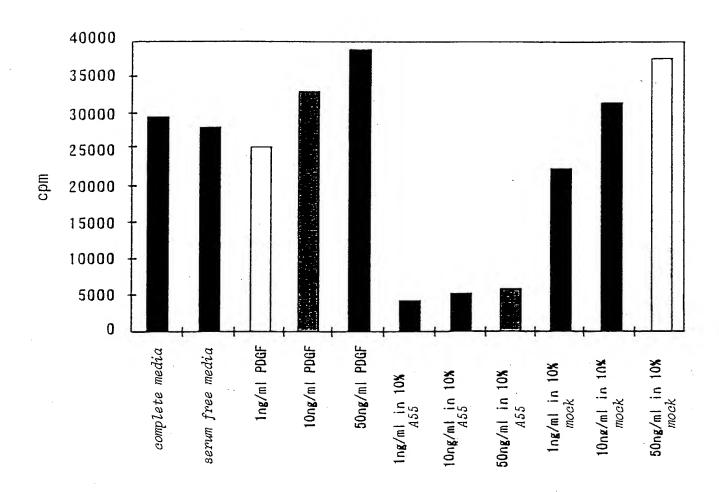


Fig. 2



09/674379 534 Rec'd PCT/PTO 30 OCT 2000

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Ile Asn Phe Arg Gly Ser Ser Val Ile Arg Leu Arg Ile Tyr Val Ser

410 415 420

cag tat ccg ttc tgagcctctg gctaaggcct ctgacactgc ctttcaccag 1458 Gln Tyr Pro Phe

425

caccgaggga cgggaggaga aaggaaacca gcaagaatga gagcgagaca gacattgcac 1518
ctttcctgct gaatatctcc tgggggcatc agcctagcat.cttgacccat atctgtacta 1578
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agtctattt aaaatcacat ctggtattt cagcataaaa gaaattttag ttgctttaa 1998
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<210> 4

<211> 423

<212> PRT

<213> Mus musculus

<400> 4

Gln Cys Thr Asn Gly Phe Asp Leu Asp Arg Gln Ser Gly Gln Cys Leu

1 5 10 15

Asp Ile Asp Glu Cys Arg Thr Ile Pro Glu Ala Cys Arg Gly Asp Met
20 25 30

Met Cys Val Asn Gln Asn Gly Gly Tyr Leu Cys Ile Pro Arg Thr Asn

35

40

45

Pro Val Tyr Arg Gly Pro Tyr Ser Asn Pro Tyr Ser Thr Ser Tyr Ser

50 55 60

Gly Pro Tyr Pro Ala Ala Ala Pro Pro Val Pro Ala Ser Asn Tyr Pro
65 70 75 80

Thr Ile Ser Arg Pro Leu Val Cys Arg Phe Gly Tyr Gln Met Asp Glu

85 90 95

Gly Asn Gln Cys Val Asp Val Asp Glu Cys Ala Thr Asp Ser His Gln
100 105 110

Cys Asn Pro Thr Gln Ile Cys Ile Asn Thr Glu Gly Gly Tyr Thr Cys
115 120 125

Ser Cys Thr Asp Gly Tyr Trp Leu Leu Glu Gly Gln Cys Leu Asp Ile 130 135 140

Asp Glu Cys Arg Tyr Gly Tyr Cys Gln Gln Leu Cys Ala Asn Val Pro

145					150					155					160
Gly	Ser	Tyr	Ser	Cys	Thr	Cys	Asn	Pro	Gly	Phe	Thr	Leu	Asn	Asp	Asp
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Gly	Arg	Ser	Cys	G1n	Asp	Val	Asn	Glu	Cys	Glu	Thr	Glu	Asn	Pro	Cys
			180					185					190		
Va1	G1n	Thr	Cys	Val	Asn	Thr	Tyr	Gly	Ser	Phe	Ile	Cys	Arg	Cys	Asp
		195					200					205			
Pro	G1y	Tyr	Glu	Leu	Glu	Glu	Asp	Gly	Ile	His	Cys	Ser	Asp	Met	Asp
	210					215					220				
Glu	Cys	Ser	Phe	Ser	Glu	Phe	Leu	Cys	Gln	His	Glu	Cys	Val	Asn	Gln
225					230					235					240
Pro	Gly	Ser	Tyr	Phe	Cys	Ser	Cys	Pro	Pro	Gly	Tyr	Val	Leu	Leu	Asp
				245					250					255	
Asp	Asn	Arg	Ser	Cys	Gln	Asp	Ile	Asn	Glu	Cys	Glu	His	Arg	Asn	His
			260					265					270		
Thr	Cys	Thr	Ser	Leu	Gln	Thr	Cys	Tyr	Asn	Leu	Gln	G1 y	Gly	Phe	Lys
		275					280					285			
Cys	Ile	Asp	Pro	Ile	Ser	Cys	Glu	Glu	Pro	Tyr	Leu	Leu	Ile	Gly	Glu
	290					295					300				
Asn	Arg	Cys	Met	Cys	Pro	Ala	Glu	His	Thr	Ser	Cys	Arg	Asp	G1n	Pro
305					310					315					320
Phe	Thr	Ile	Leu	Tyr	Arg	Asp	Met	Asp	Val	Val	Ser	G1 y	Arg	Ser	Val
				325					330	ı				335	
Pro	Ala	Asp	Ile	Phe	G1n	Met	G1n	Ala	Thr	Thr	Arg	Tyr	Pro	G1 y	Ala
			340					345					350		
Tvr	Tvr	He	Phe	Gln	He	Lvs	Ser	Glv	Asn	G1u	Gly	Arg	Glu	Phe	Tyr

355

360

365

Met Arg Gln Thr Gly Pro Ile Ser Ala Thr Leu Val Met Thr Arg Pro 370 375 380

Ile Lys Gly Pro Arg Asp Ile Gln Leu Asp Leu Glu Met Ile Thr Val
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Asn Thr Val Ile Asn Phe Arg Gly Ser Ser Val Ile Arg Leu Arg Ile
405 410 415

Tyr Val Ser Gln Tyr Pro Phe

420

<210> 5

<211> 1269

<212> DNA

<213> Mus musculus

<400> 5

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caagatgtga acgagtgcga aactgagaat ceetgtgtte agacetgtgt caacacetat 600 ggetetttea tetgeegetg tgacecagga tatgaacttg aggaagatgg catteactge 660 agtgatatgg acgagtgcag etteteegag tteetetgte aacacegagtg tgtgaaccag 720 eegggeteat acttetgete gtgeeeteea ggetaegtee tgttggatga taacegaage 780 tgeeaggata teaatgaatg tgageacega aaceacacgt gtaceteact geagacttge 840 tacaatetae aagggggett caaatgtatt gateceatea getgtgagga geettatetg 900 etgattggtg aaaacegetg tatgtgteet getgageaca eeagetgeag agaceageea 960 tteaceatee tgtateggga catggatgtg gtgteaggae geteegttee tgetgacate 1020 tteeagatge aageaacaac eegataecet ggtgeetatt acatttteea gateaaatet 1080 ggeaacegag gtegagagtt etatatgegg caaacaggge etateagtge caecetggtg 1140 atgacacgee eeateaagg geeteggac atecagetgg acttggagat gateactgte 1200 aacactgtea teaactteag aggeagetee gtgateegae tgeggatata tgtgtegeag 1260 tateegtte

<210> 6

(211) 461

<212> PRT

<213> Mus musculus

<400> 6

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-35 -30 -25

Arg Arg Met Ile Leu Thr Val Thr Ile Leu Ala Leu Trp Leu Pro His
-20 -15 -10 -5

Pro Gly Asn Ala Gln Gln Gln Cys Thr Asn Gly Phe Asp Leu Asp Arg

			-1	1				5					10		
G1n	Ser	Gly	Gln	Cys	Leu	Asp	Пe	Asp	Glu	Cys	Arg	Thr	Ile	Pro	Glu
		15					20					25			
Ala	Cys	Arg	Gly	Asp	Met	Met	Cys	Val	Asn	Gln	Asn	G1y	G1y	Tyr	Leu
	30					35					40				
Cys	He	Pro	Arg	Thr	Asn	Pro	Val	Tyr	Arg	G1y	Pro	Tyr	Ser	Asn	Pro
45					50					55					60
Tyr	Ser	Thr	Ser	Tyr	Ser	G1 y	Pro	Tyr	Pro	Ala	Ala	Ala	Pro	Pro	Va1
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Pro	Ala	Ser	Asn	Tyr	Pro	Thr	Ile	Ser	Arg	Pro	Leu	Val	Cys	Arg	Phe
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Gly	Tyr	Gln	Met	Asp	Glu	Gly	Asn	Gln	Cys	Val	Asp	Val	Asp	Glu	Cys
		95					100					105			
Ala	Thr	Asp	Ser	His	G1n	Cys	Asn	Pro	Thr	Gln	Ile	Cys	Ile	Asn	Thr
	110					115					120				
Glu	Gly	Gly	Tyr	Thr	Cys	Ser	Cys	Thr	Asp	G1y	Tyr	Trp	Leu	Leu	Glu
125					130					135					140
G1y	Gln	Cys	Leu	Asp	Ile	Asp	Glu	Cys	Arg	Tyr	Gly	Tyr	Cys	Gln	Gln
				145					150					155	
Leu	Cys	Ala	Asn	Val	Pro	Gly	Ser	Tyr	Ser	Cys	Thr	Cys	Asn	Pro	Gly
			160					165					170		
Phe	Thr	Leu	Asn	Asp	Asp	Gly	Arg	Ser	Cys	Gln	Asp	Val	Asn	Glu	Cys
		175	;				180					185			
Glu	Thr	Glu	ı Asn	Pro	Cys	Val	G1n	Thr	Cys	: Val	Asn	Thr	Tyr	G1 y	Ser
	190)				195					200				
Dlag		Cyr	. 120	Cyc	Acn	Pro	Gly	Tur	- G1 ₁	ı Lei	G1u	Glu	Asn	Glv	He

205					210					215					220
His	Cys	Ser	Asp	Met	Asp	Glu	Cys	Ser	Phe	Ser	Glu	Phe	Leu	Cys	Gln
				225					230					235	
His	Glu	Cys	Val	Asn	Gln	Pro	Gly	Ser	Tyr	Phe	Cys	Ser	Cys	Pro	Pro
			240					245					250		
G1y	Tyr	Val	Leu	Leu	Asp	Asp	Asn	Arg	Ser	Cys	Gln	Asp	He	۸sn	Glu
		255					260					265			
Cys	Glu	His	Arg	Asn	His	Thr	Cys	Thr	Ser	Leu	Gln	Thr	Cys	Tyr	Asn
	270					275					280				
Leu	Gln	G1y	G1y	Phe	Lys	Cys	Ile	Asp	Pro	Ile	Ser	Cys	Glu	Glu	Pro
285					290					295					300
Tyr	Leu	Leu	Ile	Gly	Glu	Asn	Arg	Cys	Met	Cys	Pro	Ala	Glu	His	Thr
				305					310					315	
Ser	Cys	Arg	Asp	G1n	Pro	Phe	Thr	Ile	Leu	Tyr	Arg	Asp	Met	Asp	Val
			320					325					330		
Val	Ser	G1y	Arg	Ser	Val	Pro	Ala	Asp	Ile	Phe	Gln	Met	G1n	Ala	Thr
		335					340					345			
Thr	Arg	Tyr	Pro	Gly	Ala	Tyr	Tyr	Ile	Phe	Gln	Ile	Lys	Ser	Gly	Asn
	350					355					360				
Glu	Gly	Arg	Glu	Phe	Tyr	Met	Arg	G1n	Thr	Gly	Pro	Ile	Ser	Ala	Thr
365					370					375					380
Leu	Val	Met	Thr	Arg	Pro	Ile	Lys	Gly	Pro	Arg	Asp	Ile	Gln	Leu	Asp
				385					390					395	
Leu	Glu	Met	Ile	Thr	Val	Asn	Thr	Val	Ile	Asn	Phe	Arg	Gly	Ser	Ser
			400					405					410		
Val	He	Are	Leu	Arg	He	Tvr	Va1	Ser	Gln	Tyr	Pro	Phe			

415 420 425

<210> 7

<211> 1383

<212> DNA

<213> Mus musculus

<400> 7

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<210> 8

<211> 2429

<212> DNA

<213> Mus musculus

<220>

<223> Clone mouse A55b derived from Day 13 mouse embryonic heart

<220>

<221> CDS

<222> (232).. (1614)

<220>

<221> sig_peptide

<222> (232).. (339)

<220>

<221> mat_peptide

<222> (340).. (1614)

<400> 8

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-35

cct aga agt ttc gag cca atg cac agt gga ctc tgc aga cag aga cgc 285Pro Arg Ser Phe Glu Pro Met His Ser Gly Leu Cys Arg Gln Arg Arg $-30 \qquad \qquad -25 \qquad \qquad -20$

atg ata ctc act gtt acc atc ttg gca ctc tgg ctt cca cat cct ggg 333 Met Ile Leu Thr Val Thr Ile Leu Ala Leu Trp Leu Pro His Pro Gly -15 -10 -5

aat gca cag cag tgc aca aac ggc ttt gac ctg gac cgc cag tca 381 Asn Ala Gln Gln Cys Thr Asn Gly Phe Asp Leu Asp Arg Gln Ser

-1 1 5 10

gga cag tgt cta gat att gat gaa tgc cgg acc atc cct gag gct tgt 429

Gly Gln Cys Leu Asp Ile Asp Glu Cys Arg Thr Ile Pro Glu Ala Cys

20 25 30

cgt ggg gac atg atg tgt gtc aac cag aat ggc ggg tat ttg tgc atc 477 Arg Gly Asp Met Met Cys Val Asn Gln Asn Gly Gly Tyr Leu Cys Ile

35 40 . 45

cct cga acc aac cca gtg tat cga ggg cct tac tca aat ccc tac tct 525

Pro	Arg	Thr	Asn	Pro	Val	Tyr	Arg	G1y	Pro	Tyr	Ser	Asn	Pro	Tyr	Ser	
			50					55					60			
aca	tcc	tac	tca	ggc	cca	tac	cca	gca	gcg	gcc	cca	cca	gta	cca	gct	573
Thr	Ser	Tyr	Ser	Gly	Pro	Tyr	Pro	Λla	Лlа	Ala	Pro	Pro	Val	Pro	Ala	
		65					70					75				
tcc	aac	tac	ссс	acg	att	tca	agg	cct	ctt	gtc	tgc	cgc	ttt	ggg	tat	621
Ser	Asn	Tyr	Pro	Thr	Ile	Ser	Arg	Pro	Leu	Val	Cys	Arg	Phe	G1y	Tyr	
	80					85					90					
cag	atg	gat	gaa	ggc	aac	cag	tgt	gtg	gat	gtg	gac	gag	tgt	gca	aca	669
G1n	Met	Asp	Glu	G1 y	Asn	Gln	Cys	Val	Asp	Val	Asp	Glu	Cys	Ala	Thr	
95					100	-				105					110	
gac	tca	cac	cag	tgc	aac	cct	acc	cag	atc	tgt	atc	aac	act	gaa	gga	717
Asp	Ser	His	G1n	Cys	Asn	Pro	Thr	G1n	Ile	Cys	Ile	Asn	Thr	Glu	Gly	
				115					120					125		
ggt	tac	acc	tgc	tcc	tgc	acc	gat	ggg	tac	tgg	ctt	ctg	gaa	ggg	cag	765
Gly	Tyr	Thr	Cys	Ser	Cys	Thr	Asp	G1 y	Tyr	Trp	Leu	Leu	Glu	Gly	G1n	
			130					135					140			
tgc	cta	gat	att	gat	gaa	tgt	cgc	tat	ggt	tac	tgc	cag	cag	ctc	tgt	813
Cys	Leu	Asp	Ile	Asp	Glu	Cys	Arg	Tyr	Gly	Tyr	Cys	Gln	Gln	Leu	Cys	
		145					150					155				
gca	aat	gtt	cca	gga	tcc	tat	tcc	tgt	aca	tgc	aac	cct	ggt	ttc	acc	861
Ala	Asn	Val	Pro	Gly	Ser	Tyr	Ser	Cys	Thr	Cys	Asn	Pro	G1y	Phe	Thr	
	160					165					170					
ctc	aac	gac	gat	gga	agg	tct	tgc	caa	gat	gtg	aac	gag	tgc	gaa	act	909
Leu	Asn	Asp	Asp	Gly	Arg	Ser	Cys	Gln	Asp	Val	Asn	Glu	Cys	Glu	Thr	
175					180					185					190	

gag aat ccc tgt gtt cag acc tgt gtc aac acc tat ggc tct ttc atc Glu Asn Pro Cys Val Gln Thr Cys Val Asn Thr Tyr Gly Ser Phe Ile 205 200 195 tgc cgc tgt gac cca gga tat gaa ctt gag gaa gat ggc att cac tgc 1005 Cys Arg Cys Asp Pro Gly Tyr Glu Leu Glu Glu Asp Gly Ile His Cys 220 210 215 agt gat atg gac gag tgc agc ttc tcc gag ttc ctc tgt caa cac gag 1053 Ser Asp Met Asp Glu Cys Ser Phe Ser Glu Phe Leu Cys Gln His Glu 225 230 235 tgt gtg aac cag ccg ggc tca tac ttc tgc tcg tgc cct cca ggc tac 1101 Cys Val Asn Gln Pro Gly Ser Tyr Phe Cys Ser Cys Pro Pro Gly Tyr 250 240 245 gtc ctg ttg gat gat aac cga agc tgc cag gat atc aat gaa tgt gag 1149 Val Leu Leu Asp Asp Asn Arg Ser Cys Gln Asp Ile Asn Glu Cys Glu 270 255 260 265 cac cga aac cac acg tgt acc tca ctg cag act tgc tac aat cta caa 1197 His Arg Asn His Thr Cys Thr Ser Leu Gln Thr Cys Tyr Asn Leu Gln 285 280 275 ggg ggc ttc aaa tgt att gat ccc atc agc tgt gag gag cct tat ctg 1245 Gly Gly Phe Lys Cys Ile Asp Pro Ile Ser Cys Glu Glu Pro Tyr Leu 300 290 295 ctg att ggt gaa aac cgc tgt atg tgt cct gct gag cac acc agc tgc 1293 Leu Ile Gly Glu Asn Arg Cys Met Cys Pro Ala Glu His Thr Ser Cys 305 310 315 aga gac cag cca ttc acc atc ctg tat cgg gac atg gat gtg gtg tca 1341 Arg Asp Gln Pro Phe Thr Ile Leu Tyr Arg Asp Met Asp Val Val Ser

330 325 320 gga cgc tcc gtt cct gct gac atc ttc cag atg caa gca aca acc cga 1389 Gly Arg Ser Val Pro Ala Asp Ile Phe Gln Met Gln Ala Thr Thr Arg 350 345 340 335 tac cct ggt gcc tat tac att ttc cag atc aaa tct ggc aac gag ggt 1437 Tyr Pro Gly Ala Tyr Tyr Ile Phe Gln Ile Lys Ser Gly Asn Glu Gly 365 355 360 cga gag ttc tat atg cgg caa aca ggg cct atc agt gcc acc ctg gtg 1485 Arg Glu Phe Tyr Met Arg Gln Thr Gly Pro Ile Ser Ala Thr Leu Val 380 375 370 atg aca cgc ccc atc aaa ggg cct cgg gac atc cag ctg gac ttg gag 1533 Met Thr Arg Pro Ile Lys Gly Pro Arg Asp Ile Gln Leu Asp Leu Glu 395 390 385 atg atc act gtc aac act gtc atc aac ttc aga ggc agc tcc gtg atc 1581 Met Ile Thr Val Asn Thr Val Ile Asn Phe Arg Gly Ser Ser Val Ile 410 405 400 cga ctg cgg ata tat gtg tcg cag tat ccg ttc tgagcctctg gctaaggcct 1634 Arg Leu Arg Ile Tyr Val Ser Gln Tyr Pro Phe 425 420 415 ctgacactgc ctttcaccag caccgaggga cgggaggaga aaggaaacca gcaagaatga 1694 gagcgagaca gacattgcac ctttcctgct gaatatctcc tggggggcatc agcctagcat 1754 cttgacccat atctgtacta ttgcagatgg tcactctgaa ggacaccctg ccctcagttc 1814 ctatgatgca gttatccaaa agtgttcatc ttagcccctg atatgaggtt gccagtgact 1874 cttcaaagcc ttccatttat ttccatcgtt ttataaaaaa gaaaatagat tagatttgct 1934 ggggtatgag tcctcgaagg ttcaaaagac tgagtggctt gctctcacct cttcctctcc 1994

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<210> 9

<211> 423

<212> PRT

<213> Mus musculus

<400> 9

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Asp Ile Asp Glu Cys Arg Thr Ile Pro Glu Ala Cys Arg Gly Asp Met
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Met Cys Val Asn Gln Asn Gly Gly Tyr Leu Cys Ile Pro Arg Thr Asn 35 40 45

Pro Val Tyr Arg Gly Pro Tyr Ser Asn Pro Tyr Ser Thr Ser Tyr Ser 50 55 60

Gly Pro Tyr Pro Ala Ala Ala Pro Pro Val Pro Ala Ser Asn Tyr Pro 65 70 75 80

Thr Ile Ser Arg Pro Leu Val Cys Arg Phe Gly Tyr Gln Met Asp Glu

				85					90					95	
Gly	Asn	Gln	Cys	Val	Asp	Val	Asp	Glu	Cys	Ala	Thr	Asp	Ser	His	G1n
			100					105					110		
Cys	Asn	Pro	Thr	Gln	He	Cys	Ile	Asn	Thr	Glu	Gly	Gly	Tyr	Thr	Cys
		115					120					125			
Ser	Cys	Thr	Asp	Gly	Tyr	Trp	Leu	Leu	G1u	G1 y	G1n	Cys	Leu	Asp	Ile
	130					135					140				
Asp	Glu	Cys	Arg	Tyr	Gly	Tyr	Cys	Gln	Gln	Leu	Cys	Ala	Asn	Val	Pro
145					150					155					160
G1y	Ser	Tyr	Ser	Cys	Thr	Cys	Asn	Pro	Gly	Phe	Thr	Leu	Asn	Asp	Asp
				165					170					175	
Gly	Arg	Ser	Cys	Gln	Asp	Va1	Asn	G1u	Cys	G1u	Thr	Glu	Asn	Pro	Cys
			180					185					190		
Val	G1n	Thr	Cys	Val	Asn	Thr	Tyr	Gly	Ser	Phe	He	Cys	Arg	Cys	Asp
		195					200					205			
Pro	G1y	Tyr	Glu	Leu	G1u	Glu	Asp	Gly	Ile	His	Cys	Ser	Asp	Met	Asp
	210					215					220				
G1u	Cys	Ser	Phe	Ser	Glu	Phe	Leu	Cys	Gln	His	G1u	Cys	Val	Asn	Gln
225					230					235					240
Pro	Gly	Ser	Tyr	Phe	Cys	Ser	Cys	Pro	Pro	G1y	Tyr	Val	Leu	Leu	Asp
				245					250					255	
Asp	Asn	Arg	Ser	Cys	G1n	Asp	Ile	Asn	Glu	Cys	Glu	His	Arg	Asn	His
			260					265					270		
Thr	Cys	Thr	Ser	Leu	G1n	Thr	Cys	Tyr	Asn	Leu	Gln	G1y	G1y	Phe	Lys
		275					280					285			
Cvc	110	Asn	Pro	110	Sor	Cvc	Glu	Clu	Dro	Tur	Lou	Lou	Tlo	C1v	G1n

Asn Arg Cys Met Cys Pro Ala Glu His Thr Ser Cys Arg Asp Gln Pro Phe Thr Ile Leu Tyr Arg Asp Met Asp Val Val Ser Gly Arg Ser Val Pro Ala Asp Ile Phe Gln Met Gln Ala Thr Thr Arg Tyr Pro Gly Ala Tyr Tyr Ile Phe Gln Ile Lys Ser Gly Asn Glu Gly Arg Glu Phe Tyr Met Arg Gln Thr Gly Pro Ile Ser Ala Thr Leu Val Met Thr Arg Pro Ile Lys Gly Pro Arg Asp Ile Gln Leu Asp Leu Glu Met Ile Thr Val Asn Thr Val Ile Asn Phe Arg Gly Ser Ser Val Ile Arg Leu Arg Ile Tyr Val Ser Gln Tyr Pro Phe

<210> 10

<211> 1269

<212> DNA

<213> Mus musculus

<400> 10

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<210> 11

<211> 448

<212> PRT

<213> Homo sapiens

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		-5				-1	1				5				
Leu	Asp	Arg	G1n	Ser	Gly	Gln	Cys	Leu	Asp	Ile	Asp	Glu	Cys	Arg	Thr
10					15					20	,				25
Ile	Pro	Glu	Ala	Cys	Arg	Gly	Asp	Met	Met	Cys	Val	Asn	Gln	Asn	Gly
				30					35					40	
Gly	Tyr	Leu	Cys	Ile	Pro	Arg	Thr	Asn	Pro	Val	Tyr	Arg	G1y	Pro	Tyr
			45					50					55		
Ser	Asn	Pro	Tyr	Ser	Thr	Pro	Tyr	Ser	Gly	Pro	Tyr	Pro	Ala	Ala	Ala
		60					65					70			
Pro	Pro	Leu	Ser	Ala	Pro	Asn	Tyr	Pro	Thr	Ile	Ser	Arg	Pro	Leu	He
	75					80					85				
Cys	Årg	Phe	G1y	Tyr	Gln	Met	Asp	Glu	Ser	Asn	G1n	Cys	Val	Asp	Val
90					95					100					105
Asp	Glu	Cys	Ala	Thr	Asp	Ser	His	G1n	Cys	Asn	Pro	Thr	Gln	Ile	Cys
				110					115					120	
He	Asn	Thr	Glu	G1 y	Gly	Tyr	Thr	Cys	Ser	Cys	Thr	Asp	Gly	Tyr	Trp
			125					130					135		
Leu	Leu	Glu	Gly	Ģļn	Cys	Leu	Asp	Ile	Asp	Glu	Cys	Arg	Tyr	Gly	Tyr
		140	•				145					150			

Cys Gln Gln Leu Cys Ala Asn Val Pro Gly Ser Tyr Ser Cys Thr Cys

	155					160					165				
Asn	Pro	Gly	Phe	Thr	Leu	Λsn	Glu	Asp	Gly	Arg	Ser	Cys	G1n	Asp	Val
170					175					180					185
Asn	Glu	Cys	Ala	Thr	Glu	Asn	Pro	Cys	Val	G1n	Thr	Cys	Val	Asn	Thr
				190					195					200	
Tyr	Gly	Ser	Phe	Ile	Cys	Arg	Cys	Asp	Pro	Gly	Tyr	Glu	Leu	Glu	G1u
			205					210					215		
Asp	Gly	Val	His	Cys	Ser	Asp	Met	Asp	Glu	Cys	Ser	Phe	Ser	Glu	Phe
		220					225					230			
Leu	Cys	Gln	His	Glu	Cys	Val	Asn	Gln	Pro	Gly	Thr	Tyr	Phe	Cys	Ser
	235					240					245				
Cys	Pro	Pro	Gly	Tyr	Ile	Leu	Leu	Asp	Asp	Asn	Λrg	Ser	Cys	G1n	Asp
250					255					260					265
Ile	Asn	Glu	Cys	Glu	His	Arg	Asn	His	Thr	Cys	Asn	Leu	G1n	G1n	Thr
				270					275					280	
Cys	Tyr	Asn	Leu	Gln	G1y	G1y	Phe	Lys	Cys	Ile	Asp	Pro	Ile	Arg	Cys
			285					290					295		
Glu	Glu	Pro	Tyr	Leu	Arg	Ιle	Ser	Asp	Asn	Arg	Cys	Met	Cys	Pro	Ala
		300					305					310			
Glu	Asn	Pro	Gly	Cys	Arg	Asp	Gln	Pro	Phe	Thr	Ile	Leu	Tyr	Arg	Asp
	315					320					325				
Met	Asp	Val	Val	Ser	G1y	Arg	Ser	Val	Pro	Ala	Asp	Ile	Phe	Gln	Met
330					335					340					345
GIn	Ala	Thr	Thr	Arg	Tyr	Pro	Gly	Ala	Tyr	Tyr	Ile	Phe	Gln	Ile	Lys
				350					355					360	
Ser	Gly	Asn	Glu	Gly	Arg	Glu	Phe	Tyr	Met	Arg	Gln	Thr	G1 y	Pro	He

365

370

375

Ser Ala Thr Leu Val Met Thr Arg Pro Ile Lys Gly Pro Arg Glu Ile

380 385 390

Gln Leu Asp Leu Glu Met IIe Thr Val Asn Thr Val IIe Asn Phe Arg
395 400 405

Gly Ser Ser Val Ile Arg Leu Arg Ile Tyr Val Ser Gln Tyr Pro Phe
410 415 420 425

<210> 12

<211> 1344

<212> DNA

<213> Homo sapiens

<400> 12

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gatggcgttc attgcagtga tatggacgag tgcagettet etgagtteet etgecaacat 780 gatggcgtte attgcagtga tatggacgag tgcagettet etgagtteet etgecaacat 780 gagtgtgga accagecegg cacatactte tgeteetgee etceaggeta cateetgetg 840 gatgacaace gaagetgeea agacateaac gaatgtgage acaggaacea eacgtgeaac 900 etgeageaga egtgetacaa tttacaaggg ggetteaaat geategacee eateegetgt 960 gaggageett atetgaggat eagtgataac egetgtatgt gteetgetga gaaceetgge 1020 tgcagagace ageeetttae eateetgtae egggacatgg acgtggtge aggaegetee 1080 gtteeegetg acatetteea aatgcaagee aegaeeeget aeeetgggge etattacatt 1140 tteeagatea aatetgggaa tgagggcaga gaattttaea tgeggeaaac gggeeeeate 1200 agtgeeacee tggtgatgae aegeeeeate aaagggeeee gggaaateea getggaettg 1260 gaaatgatea etgteaacae tgteateaac tteagaggea geteegtgat eegaetgeg 1320 atatatgtgt egeagtaeee atte

<210> 13

<211> 2328

<212> DNA

<213> Homo sapiens

<220>

<223> Clone human A55 derived from human brain

<220>

<221> CDS

<222> (169).. (1512)

<220>

<221> sig_peptide

<222> (169)..(237)

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<400> 13

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cegegageee egagggeeea gaggaggeeg acgtgeeega geteeteegg gggteeegee 120
cgcgagettt ettetegeet tegeatetee teeteggeg tettggae atg eea gga 177
Met Pro Gly

-23

ata aaa agg ata ctc act gtt acc att ctg gct ctc tgt ctt cca agc 225

Ile Lys Arg Ile Leu Thr Val Thr Ile Leu Ala Leu Cys Leu Pro Ser

-20 -15 -10 -5

cct ggg aat gca cag gca cag tgc acg aat ggc ttt gac ctg gat cgc 273 Pro Gly Asn Ala Gln Ala Gln Cys Thr Asn Gly Phe Asp Leu Asp Arg

-1 1 5 10

cag tca gga cag tgt tta gat att gat gaa tgc cga acc atc ccc gag 321 Gln Ser Gly Gln Cys Leu Asp Ile Asp Glu Cys Arg Thr Ile Pro Glu

15 20 25

gcc tgc cga gga gac atg atg tgt gtt aac caa aat ggc ggg tat tta 369 Ala Cys Arg Gly Asp Met Met Cys Val Asn Gln Asn Gly Gly Tyr Leu

30 35 40

tgc	att	ccc	cgg	aca	aac	cct	gtg	tat	cga	ggg	ccc	tac	tcg	aac	ccc	417
Cys	Пе	Pro	Arg	Thr	Asn	Pro	Val	Tyr	Arg	Gly	Pro	Tyr	Ser	Asn	Pro	
45					50					55					60	
tac	tcg	acc	ссс	tac	tca	ggt	ccg	tac	cca	gca	gct	gcc	cca	cca	ctc	465
Tyr	Ser	Thr	Pro	Tyr	Ser	Gly	Pro	Tyr	Pro	Ala	Ala	Ala	Pro	Pro	Leu	
				65					70					75		
tca	gct	cca	aac	tat	ссс	acg	atc	tcc	agg	cct	ctt	ata	tgc	cgc	ttt	513
Ser	Ala	Pro	Asn	Tyr	Pro	Thr	Ile	Ser	Arg	Pro	Leu	Ile	Cys	Arg	Phe	
			80					85					90			
gga	tac	cag	atg	gat	gaa	agc	aac	caa	tgt	gtg	gat	gtg	gac	gag	tgt	561
Gly	Tyr	Gln	Met	Asp	G1u	Ser	Asn	Gln	Cys	Val	Asp	Val	Asp	Glu	Cys	
		95					100					105			ı	
gca	aca	gat	tcc	cac	cag	tgc	aac	ccc	acc	cag	atc	tgc	atc	aat	act	609
Ala	Thr	Asp	Ser	His	G1n	Cys	Asn	Pro	Thr	Gln	Ile	Cys	Ile	Asn	Thr	
	110					115					120					
gaa	ggc	ggg	tac	acc	tgc	tcc	tgc	acc	gac	gga	tat	tgg	ctt	ctg	gaa	657
Glu	Gly	G1y	Tyr	Thr	Cys	Ser	Cys	Thr	Asp	Gly	Tyr	Trp	Leu	Leu	Glu	
125					130					135					140	
ggc	cag	tgc	tta	gac	att	gat	gaa	tgt	cgc	tat	ggt	tac	tgc	cag	cag	705
Gly	G1n	Cys	Leu	Asp	He	Asp	Glu	Cys	Arg	Tyr	Gly	Tyr	Cys	Gln	G1n	
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ctc	tgt	gcg	aat	gtt	cct	gga	tcc	tat	tct	tgt	aca	tgc	aac	cct	ggt	753
Leu	Cys	Ala	Asn	Val	Pro	Gly	Ser	Tyr	Ser	Cys	Thr	Cys	Asn	Pro	G1y	
			160					165					170			
ttt	acc	ctc	aat	gag	gat	gga	agg	tct	tgc	caa	gat	gtg	aac	gag	tgt	801
Phe	Thr	Leu	Asn	Glu	Asp	G1y	Arg	Ser	Cys	Gln	Asp	Val	Asn	Glu	Cys	

		175					180					185				
gcc	acc	gag	aac	ссс	tgc	gtg	caa	acc	tgc	gtc	aac	acc	tac	ggc	tct	849
Ala	Thr	Glu	Asn	Pro	Cys	Va1	Gln	Thr	Cys	Val	Asn	Thr	Tyr	Gly	Ser	
	190					195					200					
ttc	atc	tgc	cgc	tgt	gac	cca	gga	tat	gaa	ctt	gag	gaa	gat	ggc	gtt	897
Phe	He	Cys	Arg	Cys	Asp	Pro	Gly	Tyr	G1u	Leu	Glu	Glu	Asp	Gly	Val	
205					210					215					220	
cat	tgc	agt	gat	atg	gac	gag	tgc	agc	ttc	tct	gag	ttc	ctc	tgc	caa	945
His	Cys	Ser	Asp	Met	Asp	Glu	Cys	Ser	Phe	Ser	Glu	Phe	Leu	Cys	G1n	
				225					230					235		
cat	gag	tgt	gtg	aac	cag	ссс	ggc	aca	tac	ttc	tgc	tcc	tgc	cct	cca	993
His	Glu	Cys	Val	Asn	Gln	Pro	Gly	Thr	Tyr	Phe	Cys	Ser	Cys	Pro	Pro	
			240					245					250			
ggc	tac	atc	ctg	ctg	gat	gac	aac	cga	agc	tgc	caa	gac	atc	aac	gaa	1041
Gly	Tyr	Ile	Leu	Leu	Asp	Asp	Asn	Arg	Ser	Cys	G1n	Asp	Ile	Asn	G1u	
		255					260					265				
tgt	gag	cac	agg	aac	cac	acg	tgc	aac	ctg	cag	cag	acg	tgc	tac	aat	1089
Cys	Glu	His	Arg	Asn	His	Thr	Cys	Asn	Leu	Gln	Gln	Thr	Cys	Tyr	Asn	
	270					275					280					
tta	caa	ggg	ggc	ttc	aaa	tgc	atc	gac	ccc	atc	cgc	tgt	gag	gag	cct	1137
Leu	G1n	G1y	Gly	Phe	Lys	Cys	Ιlе	Asp	Pro	Ile	Arg	Cys	Glu	Glu	Pro	
285					290					295					300	
tat	ctg	agg	atc	agt	gat	aac	cgc	tgt	atg	tgt	cct	gct	gag	aac	cct	1185
Tyr	Leu	Arg	Пe	Ser	Asp	Asn	Arg	Cys	Met	Cys	Pro	Ala	Glu	Asn	Pro	
				305					310					315		
ggc	tgc	aga	gac	cag	ссс	ttt	acc	atc	ttg	tac	cgg	gac	atg	gac	gtg	1233

Gly Cys Arg Asp Gln Pro Phe Thr Ile Leu Tyr Arg Asp Met Asp Val 320 325 330 gtg tca gga cgc tcc gtt ccc gct gac atc ttc caa atg caa gcc acg 1281 Val Ser Gly Arg Ser Val Pro Ala Asp Ile Phe Gln Met Gln Ala Thr 340 335 345 acc ege tae eet ggg gee tat tae att tte eag ate aaa tet ggg aat 1329 Thr Arg Tyr Pro Gly Ala Tyr Tyr Ile Phe Gln Ile Lys Ser Gly Asn 350 355 360 gag ggc aga gaa ttt tac atg cgg caa acg ggc ccc atc agt gcc acc 1377 Glu Gly Arg Glu Phe Tyr Met Arg Gln Thr Gly Pro Ile Ser Ala Thr 365 370 375 380 ctg gtg atg aca cgc ccc atc aaa ggg ccc cgg gaa atc cag ctg gac 1425 Leu Val Met Thr Arg Pro Ile Lys Gly Pro Arg Glu Ile Gln Leu Asp 385 390 395 ttg gaa atg atc act gtc aac act gtc atc aac ttc aga ggc agc tcc 1473 Leu Glu Met Ile Thr Val Asn Thr Val Ile Asn Phe Arg Gly Ser Ser 400 405 gtg atc cga ctg cgg ata tat gtg tcg cag tac cca ttc tgagcctcgg 1522 Val Ile Arg Leu Arg Ile Tyr Val Ser Gln Tyr Pro Phe 420 425 415 gctggagcct ccgacgctgc ctctcattgg caccaaggga caggagaaga gaggaaataa 1582 cagagagaat gagagegaca cagaegttag geattteetg etgaaegttt eeeegaagag 1642 tcagccccga cttcctgact ctcacctgta ctattgcaga cctgtcaccc tgcaggactt 1702 gccacccca gttcctatga tacagttatc aaaaagtatt atcattgctc ccctgataga 1762 agattgttgg tgaattttca aggccttcag tttatttcca ctattttcaa agaaaataga 1822 ttaggtttgc gggggtctga gtctatgttc aaagactgtg aacagcttgc tgtcacttct 1882

teacetette cacteettet eteactgtgt tactgetttg caaagaeeeg ggagetggeg 1942 gggaaccetg ggagtageta gtttgetttt tgegtaeaea gagaaggeta tgtaaacaaa 2002 ccacagcagg atcgaagggt tittagagaa tgigtitcaa aaccaigcci ggiatittica 2062 accataaaag aagttteagt tgteettaaa tttgtataac ggtttaatte tgtettgtte 2122 attttgagta tttttaaaaa atatgtegta gaatteette gaaaggeett eagacacatg 2182 ctatgttetg tetteceaaa eeeagtetee teteeatttt ageeeagtgt tttetttgag 2242 gacccettaa tettgettte tttagaattt ttacccaatt ggattggaat geagaggtet 2302 2328 ccaaactgat taaatatttg aagaga

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<211> 423

<212> PRT

<213> Homo sapiens

<400> 14

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25

Met Cys Val Asn Gln Asn Gly Gly Tyr Leu Cys Ile Pro Arg Thr Asn

35 40 45

Pro Val Tyr Arg Gly Pro Tyr Ser Asn Pro Tyr Ser Thr Pro Tyr Ser

50 55 60

Gly Pro Tyr Pro Ala Ala Ala Pro Pro Leu Ser Ala Pro Asn Tyr Pro

70 75 80 65

Thr	He	Ser	Arg	Pro	Leu	Ile	Cys	Arg	Phe	Gly	Tyr	Gln	Met	Asp	Glu
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Ser	Asn	G1n	Cys	Val	Asp	Val	Λsp	Glu	Cys	Λ1а	Thr	Asp	Ser	His	Gln
			100					105					110		
Cys	Asn	Pro	Thr	Gln	Ile	Cys	Ile	Asn	Thr	Glu	Gly	G1 y	Tyr	Thr	Cys
		115					120					125			
Ser	Cys	Thr	Asp	Gly	Tyr	Trp	Leu	Leu	Glu	Gly	Gln	Cys	Leu	Asp	Ile
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Gly	Ser	Tyr	Ser	Cys	Thr	Cys	Asn	Pro	Gly	Phe	Thr	Leu	Asn	Glu	Asp
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G1y	Arg	Ser	Cys	G1n	Asp	Val	Asn	Glu	Cys	Ala	Thr	Glu	Asn	Pro	Cys
			180					185					190		
Val	Gln	Thr	Cys	Va1	Asn	Thr	Tyr	Gly	Ser	Phe	Ile	Cys	Arg	Cys	Asp
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	210)				215	;				220				
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225	5				230)				235					240
Pro	Gly	y Thi	тул	r Phe	e Cys	Ser	Cys	Pro	Pro	Gly	Tyr	· Ile	e Leu	Leu	Asp
				245	ō				250)				255	
Asp	a Ası	n Ar	g Sei	r Cys	s Glr	ı Asp	o Ile	e Asn	Glu	ı Cys	Glu	His	s Arg	, Asr	His
			260	0				265	5				270)	
Thi	c Cy:	s Ası	n Lei	u Gli	n Glr	n Thi	c Cys	s Tyr	· Asr	n Lei	ı Glr	n Gly	y Gly	Phe	e Lys
		27	5				280)				289	5		

Cys Ile Asp Pro Ile Arg Cys Glu Glu Pro Tyr Leu Arg Ile Ser Asp Asn Arg Cys Met Cys Pro Ala Glu Asn Pro Gly Cys Arg Asp Gln Pro Phe Thr Ile Leu Tyr Arg Asp Met Asp Val Val Ser Gly Arg Ser Val Pro Ala Asp Ile Phe Gln Met Gln Ala Thr Thr Arg Tyr Pro Gly Ala Tyr Tyr Ile Phe Gln Ile Lys Ser Gly Asn Glu Gly Arg Glu Phe Tyr Met Arg Gln Thr Gly Pro Ile Ser Ala Thr Leu Val Met Thr Arg Pro He Lys Gly Pro Arg Glu He Gln Leu Asp Leu Glu Met He Thr Val Asn Thr Val Ile Asn Phe Arg Gly Ser Ser Val Ile Arg Leu Arg Ile Tyr Val Ser Gln Tyr Pro Phe

<210> 15

<211> 1269

<212> DNA

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<400> 15

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096/43/9 012502

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SOLE/JOINT 1,2,3912

DECLARATION AND POWER OF ATTORNEY

I/We as below named inventors, I/We hereby declare that our residence, mailing addresses and citizenship are as stated below next to my name: that verily believe I/We are the original, inventors (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter claimed and for which a patent is sought in the application entitled:

(ii piurai names arc nsu	ed below) of the subject matter	cianned and for which	i a patent is soug	nt in the application er	intied:
	POLYPIONDE CONA EN	CODING THE POLY	YPEPTIDE AND	UTILIZATION THE	REOF
Which application is: ☐ The attached	application (A)		☑ Applicat	ion No. 09/674,379	
(for original application	JAN 2 5 2002			er 30, 2000 as the U.S.	national stage of
(var eriginal approximate	1) B JAN 2 5 2002			2284, filed April 28, 1	
	JAN 2 5 2002			,,,	
	PARTIE BY		(for declarat	ion not accompanying	application)
amended by any amend material to the patentab States Code §119(a)-(d provisional application(States of America, liste	nd understand the contents of ment referred to above; that I a ility of this application as defined) or §365(b) of any foreign as s), or §365(a) of any PCT Intered below and have also identification having a filing date before	acknowledge my duty ed in 37 C.F.R. 1.56, pplication(s) for pater national application w ied below any foreigr	to disclose informathat I hereby claimed in the or inventor's control designated in application for	mation of which I am a m priority benefits und certificate, §119(e) of at least one country of patent or inventor's co	aware and which is der Title 35, United any United States her than the United
Application Number	Country	Filio	g Date	Priority Cl Yes	laimed No
Hei. 10-119731	Japanese		28, 1998	<u>- 163</u>	
1161. 10-119731	Japanese	April	28, 1998	₩.	
International application application is not discl paragraph of Title 35, U	benefit under 35 United Standesignating the United States losed in a listed prior United United States Code, §112, I acknowled in 37 C.F.R. 1.56 which ocof this application:	s, listed below and, in States or PCT Interr mowledge my duty to	nsofar as the sub national applicati o disclose any int	ject matter of each of on in the manner pro formation material to	the claims of this ovided by the first the patentability of
Applicatio	n No.	Filing Date		Status	
<i>3)</i>					
Darryl Mexic, Reg. No. Waddell A. Biggart, Reg. 28,703; John R. Inge, F. Turner, Reg. No. 29,71 31,333; Gordon Kit, Reg. 32,197; William H. Ma Kramer, Reg. No. 33,72 George F. Lehnigk, Reg. No. 38,551 and Ecthe Patent and Trademark.	ohn H. Mion, Reg. No. 18,879; 23,063; Robert V. Sloan, Reg. g. No. 24,861; Louis Gubinsky, Reg. No. 26,916; Joseph J. Ru 0; Howard L. Bernstein, Reg. g. No. 30,764; Susan J. Mack, Indir, Reg. No. 32,156; Brian V. S.; Paul F. Neils, Reg. No. 33,1 g. No. 36,359, John T. Callaha dward F. Kenehan, Reg. No. 28 ark Office connected therewith ZINN, MACPEAK & SEAS	No. 22,775; Peter D., Reg. No. 24,835; Ne ch, Jr., Reg. No. 26,65; Alan J. F. Reg. No. 30,951; Fram W. Hannon, Reg. No. 102; Brett S. Sylvester In, Reg. No. 32,607, S. 3,962, my attorneys to h, and request that all	Olexy, Reg. No. 517; Sheldon I. I Casper, Reg. No. 18, 177; Sheldon I. I Casper, Reg. No. 18, 1778; Abraham, Reg. No. 18, 1778; Steven M. Gruski oprosecute this all correspondence	24,513; J. Frank Osha. No. 25,200; David J. Landsman, Reg. No. 2, 25,426; Kenneth J. B. Reg. No. 31,484; Mark m J. Rosner, Reg. No. 5; Robert M. Masters in, Reg. No. 36,818, Explication and to transe about the applicatio	c, Reg. No. 24.625; Cushing, Reg. No. 25,430; Richard C. Burchfiel, Reg. No. 25,430; Bruce E. 33,276; Bruce E. 4, Reg. No. 35,603, Peter A. McKenna, sact all business in the addressed to
belief are believed to be so made are punishable	tt all statements made herein of true; and further that these stat by fine or imprisonment, or h may jeopardize the validity of t	ements were made wi both, under Section 19	th the knowledge 001 of Title 18 o	that willful false state of the United States C	ments and the like
Date		First Inventor	Tasuku	HO	NJO.
-00			First Name	Middle Initial Las	st Name
Residence Kyoto	Japan J	PX Signatur	e		
City	State/Country				
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Citizenship Japanes					
Japanes	,				

		,		Second Inve	-	st Name_	Middle Initial	TASHIRO Last Name
Residence	Kyoto_ City		Japan J State/Country		Signature	_Fei	las Prince	}-
Mailing Add	ress:	93, Hiers	6 /	yama, Kita-ku, l	Kyoto-shi,	KYOTO 603	3-8162 JAPAN	
Citizenship	Japanese							
Date		A TRADE	MARKOR	Third Inven	tor <u>T</u> a	moyuki st Name	Middle Initial	NAKAMURA Last Name
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Mailing Addı	ress:	7665, Palm	ira Dr., #5324	San Diego, CA	<u> </u>			
Citizenship	Japanese							
Date				Fourth Inver	ntor <u>Ke</u>	nneth		JACOBS
Residence	Reading, M		U.S.A. State/Country	S	ignature	st Name	Middle Initial	Last Name
Residence	Reading, M City	Α	U.S.A. State/Country	S	ignature			
-	Reading, M City	Α	U.S.A. State/Country	S	ignature			
Residence Mailing Addr Citizenship	Reading, M City	A 151 Beaum	U.S.A. State/Country ont Avenue, R	S	ignature 867 or XJol	ın M.		McCoy
Residence Mailing Addr Citizenship Date	Reading, M City	A 151 Beaum	U.S.A. State/Country ont Avenue, R	eading, MA 01 Fifth Inventors	ignature 867 or XJol	nn M. 1 Name		McCoy Last Name
Residence Mailing Addr	Reading, M City ress: U.S. Reading, M City	A 151 Beaum	U.S.A. State/Country ont Avenue, R	seading, MA 01 Fifth Invento	ignature 867 or Jol Firs	nn M. 1 Name	Middle Initial	McCoy Last Name
Residence Mailing Addr Citizenship Date Residence	Reading, M City ress: U.S. Reading, M City	A 151 Beaum	U.S.A. State/Country ont Avenue, R. U.S.A. State/Country	seading, MA 01 Fifth Invento	ignature 867 or Jol Firs	nn M. 1 Name	Middle Initial	McCoy Last Name
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Residence Mailing Addr Citizenship Date Residence Mailing Addre Citizenship	Reading, M City Tess: U.S. Reading, M City ess:	A 151 Beaum A 56 Howard	U.S.A. State/Country ont Avenue, R. U.S.A. State/Country	Seading, MA 01 Fifth Invento S, MA 01867 Sixth Invento	ignature 867 or Jol Firs ignature or Julis	ın M. ıt Name	Middle Initial	McCoy Last Name

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Date				Seventh In	nventor	Edward R.		LaVallie
				_	1	First Name	Middle Initial	Last Name
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	City		State/Country					
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			1095	· · · · · · · · · · · · · · · · · ·				
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		JAN 2	5 200Z H					
Date		種	4	Eighth Inv	entor	Maurice		Treacy
	/ 	TRAD	EMARK	_ 3		First Name	Middle Initial	Last Name
Residence	Dun	Laoghaire,	IRELAND		Signat	ure		
	County o	Dublin	State/Country		لعمر	~		
	City		State/Country		ŕ	-		
Mailing Add	lress:	38 Clarino	la Park East, Dun	Laoghaire,	County	Dublin, IRELA	ND	
Citimanalia	IDEL 4.							
Citizenship	IRELAN	ID .						
Date				Ninth Inve	ntor	Cheryl		Evans
				•		First Name	Middle Initial	Last Name
Residence	Germanto	wn, MD	U.S.A.	······································	Signati	ur \$ 0		
	City		State/Country					
Mailing Add	ress:	_19326 Gol	den Meadow Dri	ve, Germant	own, M	ID 20876		•
O								
Citizenship	United K	ingdom						
Date				Tenth Inve	ntor	Michael J.		Agostino
						First Name	Middle Initial	Last Name
Residence	Andover,	MA	U.S.A.		Signati	11/2	·	
	City		State/Country			/		
Mailing Addr	ress:	26 Wolcot	Avenue, Andove	er, MA 0181	10			
							7/4	-
Citizenship	U.S.						-	
Date				Eleventh		Zhijian		т.
				Inventor		Znijian		Lu
						First Name	Middle Initial	Last Name
Residence	Bedford, N	ЛA	U.S.A.		Signatu	řé		
	City		State/Country		/			
Mailing Addr	ess:	_120 Old Bu	ırlington Road, B	edford, MA	01730			
						•		
Citizenship	People's	Rep. Of Chin	a					

SOLE/JOINT

DECLARATION AND POWER OF ATTORNEY

4,67 \$10,11

I/We as below named inventors, I/We hereby declare that our residence, mailing addresses and citizenship are as stated below next to my name: that verily believe I/We are the original, inventors (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter claimed and for which a patent is sought in the application entitled:

A NOVEL POLYMENTIDE CONA ENCODING THE POLYMENTIDE AND LITTLIZATION THEREOF

Which application is:	PEPTIDE, A CONA ENCODIN	G THE POLYPEPTIDE A	ND UTILIZATION THEREOF
Which application is: The attached application (for original application)	tional 2 5 man of	☑ Appli	cation No. 09/674,379
(for original application)	JAN 2 5 2002: W		ober 30, 2000 as the U.S. national stage of
臣	A STATE OF S		9/02284, filed April 28, 1999
×	er at		
	MACEMAN		aration not accompanying application)
If we have reviewed and unde	istand the contents of the spec	ification of the above-ide	ntified application, including the claims, as
material to the patentability of t	his application as defined in 37	C F R 1.56 that I hereby	formation of which I am aware and which is claim priority benefits under Title 35, United
States Code \$119(a)-(d) or \$3	65(b) of any foreign application	n(s) for patent or inventor	's certificate, §119(e) of any United States
provisional application(s), or §3	365(a) of any PCT International	application which designat	ed at least one country other than the United
States of America, listed below	v and have also identified belov	v any foreign application	for patent or inventor's certificate or of any
PCT International application h	aving a filing date before that of	the application on which p	priority is claimed:
	*		Priority Claimed
Application Number	Country	Filing Date	Yes No
Hei. 10-119731	Japanese	April 28, 1998	
	-		
International application design application is not disclosed in paragraph of Title 35, United S	nating the United States, listed a listed prior United States of states Code, §112, I acknowledge of C.F.R. 1.56 which occurred by	pelow and, insofar as the r PCT International appli- ge my duty to disclose any	tes application(s), or §365(c) of any PCT subject matter of each of the claims of this cation in the manner provided by the first information material to the patentability of the prior application and the national or PCT
Application No.	F	iling Date	Status
Darryl Mexic, Reg. No. 23,063; Waddell A. Biggart, Reg. No. 28,703; John R. Inge, Reg. No. Turner, Reg. No. 29,710; Howaddell, St. Walliam H. Mandir, Reg. No. 33,197; William H. Mandir, Reg. No. 33,725; Paul George F. Lehnigk, Reg. No. 3 Reg. No. 38,551 and Edward F the Patent and Trademark Offi SUGHRUE, MION, ZINN,	Robert V. Sloan, Reg. No. 22,74,861; Louis Gubinsky, Reg. No. 26,916; Joseph J. Ruch, Jr., Fard L. Bernstein, Reg. No. 25,60,764; Susan J. Mack, Reg. No. 25, Eg. No. 32,156; Brian W. Hann F. Neils, Reg. No. 33,102; Bret 6,359, John T. Callahan, Reg. No. 28,962, mice connected therewith, and remarks and re	75; Peter D. Olexy, Reg. No. 24,835; Neil B. Siegel, F. Reg. No. 26,577; Sheldon 65; Alan J. Kasper, Reg. No. 30,951; Frank L. Bernstei on, Reg. No. 32,778; Abrat S. Sylvester, Reg. No. 32,607, Steven M. Gray attorneys to prosecute the equest that all corresponder, 2100 Pennsylvania Aven	2,292; Robert J. Seas, Jr., Reg. No. 21,092; No. 24,513; J. Frank Osha, Reg. No. 24,625; Reg. No. 25,200; David J. Cushing, Reg. No. I. Landsman, Reg. No. 25,430; Richard C. No. 25,426; Kenneth J. Burchfiel, Reg. No. II. Reg. No. 31,484; Mark Boland, Reg. No. II. Rosner, Reg. No. 33,276; Bruce E. 765; Robert M. Masters, Reg. No. 35,603, Inskin, Reg. No. 36,818, Peter A. McKenna, is application and to transact all business in tence about the application be addressed to the N.W., Washington, D.C. 20037-3213.
belief are believed to be true; an so made are punishable by fine	d further that these statements v	vere made with the knowle er Section 1001 of Title	hat all statements made on information and dge that willful false statements and the like 8 of the United States Code and that such thereon.
Date	First I	rventor <u>Tasuku</u>	HONJO
n		First Name	Middle Initial Last Name
Residence Kyoto	Japan	Signature	8/5/1
City	State/Country		Y
Mailing Address: 19-	4, Ohsagi-cho, Iwakura, Sakyo-l	tu, Kyoto-shi, KYOTO 60	5-0001 JAPAN
Citizenship Japanese		***	

Date					First Name	Middle Initial	Last Name
Residence	Kyoto City	Japan State/	Country	Signat	ure <u>Fei</u>	Carbino	-
Mailing Addr		93, Higashiohno-c		Kita-ku, Kyoto	o-shi, KYOTO 60	3-8162 JAPAN	
Citizenship	Japanese	JAN 2 5 2000	2Cor 3				
Date \		RACEMARY	Th	ird Inventor	Tomoyuki	Middle Initial	NAKAMURA Last Name
Residence	San Diego, City	California U.S.A	Country	Signat	ture Jon	1 hohm	
Mailing Add	ress:	7665, Palmira Dr.	#5324, San I	Diego, CA			
Citizenship	Japanese					<u></u>	
_			Fo	urth Inventor	() Kenneth		JACOBS Last Name
Date					Time M		
Residence	Reading, M	A U.S.A		Signa		Middle Initial	-
-W	Reading, M	A U.S.A	 Country	Signa	ture <u>Kenne</u>	Middle Initial	-
Residence	Reading, M	A U.S.A State/	 Country <u>Naud</u> enue, Readin	Signa Signa	ture <u>Kenne</u>	eth frede	
Residence Mailing Add	Reading, M City ress:	A U.S.A State/	 Country <i>New Li</i> enue, Readin	Signa Signa	ture Kenne	eth feide	McCoy
Residence Mailing Add Citizenship	Reading, M City ress:	State/ State/ 151 Beaumont Av	Country Nowline enue, Readin	Signa OLY 66 g, MA 01867	First Name ture Kenne Sty MA John M. First Name	eth frede	McCoy Last Name
Residence Mailing Add Citizenship Date	Reading, M City ress: U.S. Reading, M City	State/ State/ 151 Beaumont Av	Country Mouli enue, Readin Fi	Signa Signa 91867 fth Inventor Signa	First Name ture Kenne Sty MA John M. First Name	Middle Initial	McCoy Last Name
Residence Mailing Add Citizenship Date Residence	Reading, M City ress: U.S. Reading, M City	State/ IS U.S.A State/ IS Beaumont Av IA U.S.A State/	Country Mouli enue, Readin Fi	Signa Signa 91867 fth Inventor Signa	First Name ture Kenne Sty MA John M. First Name	Middle Initial	McCoy Last Name
Residence Mailing Add Citizenship Date Residence Mailing Add	Reading, M City ress: U.S. Reading, M City ress:	State/ IS U.S.A State/ IS Beaumont Av IA U.S.A State/	Country Machine Readin Fi Country Reading, Ma	Signa Signa 91867 fth Inventor Signa	First Name ture Kenne Sty MA John M. First Name	Middle Initial	McCoy Last Name
Residence Mailing Add Citizenship Date Residence Mailing Add Citizenship	Reading, M City ress: U.S. Reading, M City ress:	IA U.S.A State/ IS1 Beaumont Av IA U.S.A State/ State/ State/ U.S.A U.S.A	Country Pauli Readin Fi Country Reading, Ma	Signa LY66 g, MA 01867 fth Inventor Signa A 01867	John M. First Name ture Lisa A. First Name	Middle Initial	McCoy Last Name Collins-Racie

13671379 012502

Date		Twelfth I	nventor David		Merberg	
Residence Acton, N		U.S.A.	First Name Signature	Middle Initial	Last Name	
	City	State/Country			•	
Mailing Address:		2 Orchard Drive, Acton, MA 01720				
Citizenship	U.S.					

DECLARATION AND POWER OF ATTORNEY

I/We as below named inventors, I/We hereby declare that our residence, mailing addresses and citizenship are as stated below next to my name: that verily believe I/We are the original; inventors (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter claimed and for which a patent is sought in the application entitled:

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		PNA ENCODING THE P	OLYPEPTIDE AND UTI	LIZATION THEREOF
Which application is The attach		3095	☑ Application N	Jo. 00/674 370
(for original applicat		2002 OT		, 2000 as the U.S. national stage of
(.o. og app	JAN: 25	2002 <u></u>		I, filed April 28, 1999
	JAN	₽ .	-	
	V .	ASS	(for declaration r	not accompanying application)
				application, including the claims,
				on of which I am aware and which iority benefits under Title 35, Unit
				ficate, §119(e) of any United Stat
				ast one country other than the Unit
States of America, I	isted below and have als	o identified below any for	reign application for pate	nt or inventor's certificate or of a
PCT International ap	plication having a filing o	date before that of the appl	ication on which priority i	is claimed:
·				Priority Claimed
Application Numb	per Co	ountry	Filing Date	Yes No
Hei. 10-119731	Ja	panese A	April 28, 1998	∅ 🗆
International applica application is not di paragraph of Title 3: this application as de	tion designating the Unit isclosed in a listed prior 5, United States Code, §1	ted States, listed below an United States or PCT I 112, I acknowledge my du	id, insofar as the subject international application in ity to disclose any inform	dication(s), or §365(c) of any PC matter of each of the claims of the nature provided by the fination material to the patentability application and the national or PC
_		ru n		
Applic	ation No.	Filing Date		Status
Darryl Mexic, Reg. N. Waddell A. Biggart, 28,703; John R. Ing. Turner, Reg. No. 29 31,333; Gordon Kit, 32,197; William H. Kramer, Reg. No. 33 George F. Lehnigk, Reg. No. 38,551 and the Patent and Trad	No. 23,063; Robert V. Slo Reg. No. 24,861; Louis Ce, Reg. No. 26,916; Jose ,710; Howard L. Bernste Reg. No. 30,764; Susan . Mandir, Reg. No. 32,156 ,725; Paul F. Neils, Reg. Reg. No. 36,359, John T Edward F. Kenehan, Re emark Office connected	pan, Reg. No. 22,775; Pete Gubinsky, Reg. No. 24,835 ph J. Ruch, Jr., Reg. No. in, Reg. No. 25,665; Alar J. Mack, Reg. No. 30,951; S. Brian W. Hannon, Reg. No. 33,102; Brett S. Sylv Callahan, Reg. No. 32,6 g. No. 28,962, my attorne therewith, and request th	r D. Olexy, Reg. No. 24,5; Neil B. Siegel, Reg. No. 26,577; Sheldon I. Land J. Kasper, Reg. No. 25,4; Frank L. Bernstein, Reg. No. 32,778; Abraham J. ester, Reg. No. 32,765; F07, Steven M. Gruskin, Rys to prosecute this applicat all correspondence about the steries of the ste	Robert J. Seas, Jr., Reg. No. 21,09 (513; J. Frank Osha, Reg. No. 24,62 (25,200; David J. Cushing, Reg. No. 18man, Reg. No. 25,430; Richard (426; Kenneth J. Burchfiel, Reg. No. 31,484; Mark Boland, Reg. No. 30,276; Bruce Robert M. Masters, Reg. No. 35,60 (19), No. 36,818, Peter A. McKenn cation and to transact all business out the application be addressed V., Washington, D.C. 20037-3213.
belief are believed to so made are punisha	be true; and further that ble by fine or imprison:	these statements were mad	le with the knowledge that on 1001 of Title 18 of th	statements made on information as t willful false statements and the li- ne United States Code and that suc 1.
Date		First Inventor	Tasuku	ОГИОН
D 1	.	2:		Middle Initial Last Name
Residence Kyot		iSig: /Country	nature	
City	State	Cominy	<u> </u>	
Mailing Address:	19-4, Ohsagi-cho,	, Iwakura, Sakyo-ku, Kyot	o-shi, KYOTO 606-0001	JAPAN
	·			
Citizenship Japa	anese			
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Date			Seco	and Inventor	Kei		12:11 1:::1	TASHIRO
				G: .	First Name	7/	Middle Initial	Last Name
Residence	Kyoto		n /C	Signati	ие — 7	ter-	Cashino	
	City	State	:/Country					
Mailing Addı	ress:	93, Higashiohno-	cho, Koyama, K	ita-ku, Kyoto-	shi, KYO	TO 603-	8162 JAPAN	
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Citizenship	Japanese							
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Data			Thir	d Inventor	Tomoyu	ıki		NAKAMURA
Date					First Nam	ie	Middle Initial	Last Name
Residence	San Diego,	California U.S.	A	Signati	$I_{\text{ire}} = I$	onli	nohn	
	City	State	e/Country		1			
A		7665, Palmira D	r #5324 San Di	iego CA				
Mailing Add	1622:	7005, ranima D	1., #JJZ4, San Di					
	-							
Citizenship	Japanese							
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		1			O V a1			JACOBS
Date			Fou	rth Inventor	First Nam	ne	Middle Initial	Last Name
Residence	Reading, M	IA U.S	.A.	Signat				
COMMENT	City		e/Country					
	-		B 27	MA 01067				
Mailing Add	ress:	151 Beaumont A	venue, Keading,	, MA 0186/				
Citizenship	U.S.							
Chizenship	0.3.							
~()	11/28/01				~			MaCan
Date	11 0401		Fift	h Inventor	John M First Nan		Middle Initial	_McCoy Last Name
Residence	Reading, M	IA U.S	.A	Signat	,	" / ₈ X	~ hibic	_
Residence	City		e/Country					
				m	1	- /		
Mailing Add	iress:	56 Howard Stre	et, <u>Reading</u> , MA	01867 717	7			
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G1: 1.1	** **							
Citizenship	U.K.				-			

Date			Six	th Inventor	Lisa A.		Adidate februar	Collins-Racie
		***		Ciarra	First Nar	ne	Middle Initial	Last Name
Residence	Acton, MA		s.A. te/Country	Signa				
	City	Sta	country					
Mailing Ado	dress:	124 School Stre	et, Acton, MA)1720				
Citizenship	U.S.							

Date				Seventh I	nventor	Edward R.		LaVallie
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Citizenship	U.S.							
Date						Maurice First Name		Treacy
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Mailing Addi	ress:	38 Clarinda	a Park East, Dun	Laoghaire,	County	Dublin, IRELAN	D	
Citizenship	IRELAN	D						
Date				Ninth Inve	entor	Cheryl First Name	Middle Initial	Evans Last Name
Residence	Germantov City	wn, MD	U.S.A. State/Country		Signatu			
Mailing Addr	ress:	19326 Gold	den Meadow Dri	ve, Germant	town, M	D 20876		
Citizenship	United Ki	ngdom						
Date	· · · · · · · · · · · · · · · · · · ·			Tenth Inve	entor	Michael J. First Name	Middle Initial	Agostino Last Name
Residence	Andover, N	Л А	U.S.A. State/Country		Signatu /			Last Ivanic
Mailing Addr	ess:	26 Wolcott	Avenue, Andove	er, MA 018	10			
Citizenship	U.S.							
Date				Eleventh Inventor		Zhijian		Lu
Residence	Bedford, M	IA .	U.S.A. State/Country		Signatu	First Name	Middle Initial	Last Name
Mailing Addr	-	120 Old Bu	rlington Road, B	edford, MA	01730			
Citizenshin	Poomlois F				· · · -			

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Date		Twelfth I	nventor David	Merberg
Residence	Acton, MA	U.S.A.	First Name Signature	Middle Initial Last Name
	City	State/Country?	/	
Mailing Address:		2 Orchard Drive, Acton, MA 01720		And the second s
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Citizenship	U.S.	•		



DECLARATION AND POWER OF ATTORNEY

I/We as below named inventors, I/We hereby declare that our residence, mailing addresses and citizenship are as stated below next to my name: that verily believe I/We are the original, inventors (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter claimed and for which a patent is sought in the application entitled:

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_ * *			cation No. 09/674,379	
The attached applicati (for original application)			ober 30, 2000 as the U.S.	national stage of
(for original application)	JAN 2 5 2002 W		0/02284, filed April 28, 1	
	JAN 2 5 2002			
I/We have reviewed and under	The state of the s	(for declar	ration not accompanying	g application)
I/We have reviewed and under- amended by any amendment ref- material to the patentability of th	erred to above; that I acknow	ledge my duty to disclose int	formation of which I am	aware and which is
States Code §119(a)-(d) or §36	5(b) of any foreign applicati	on(s) for patent or inventor's	s certificate, §119(e) of	f any United States
provisional application(s), or §3	65(a) of any PCT Internationa	I application which designate	ed at least one country of	ther than the United
States of America, listed below	and have also identified belo	ow any foreign application for	or patent or inventor's c	certificate or of any
PCT International application ha	ving a filing date before that	of the application on which pr		
Application Number	Country	Filing Date	Priority C Yes	Claimed No
Hei. 10-119731	Japanese	April 28, 1998	<u></u>	
Hei. 10-119731	Japanese	11p111 20, 1770		_
International application designapplication is not disclosed in paragraph of Title 35, United Statistics application as defined in 37 international filing date of this a	a listed prior United States ates Code, §112, I acknowle C.F.R. 1.56 which occurred	or PCT International applic dge my duty to disclose any	cation in the manner pr information material to	ovided by the first the patentability of
Application No.		Filing Date	Status	
I/We hereby appoint John H. M. Darryl Mexic, Reg. No. 23,063; Waddell A. Biggart, Reg. No. 28,703; John R. Inge, Reg. No. Turner, Reg. No. 29,710; Howa 31,333; Gordon Kit, Reg. No. 3 32,197; William H. Mandir, Rekramer, Reg. No. 33,725; Paul George F. Lehnigk, Reg. No. 3 Reg. No. 38,551 and Edward F. the Patent and Trademark Offic SUGHRUE, MION, ZINN, If/We hereby declare that all stabelief are believed to be true; and so made are punishable by fine	Robert V. Sloan, Reg. No. 22, 4,861; Louis Gubinsky, Reg. 26,916; Joseph J. Ruch, Jr. and L. Bernstein, Reg. No. 25, 0,764; Susan J. Mack, Reg. Ng. No. 32,156; Brian W. Har F. Neils, Reg. No. 33,102; Brian G. 359, John T. Callahan, Reg. Kenehan, Reg. No. 28,962, and MACPEAK & SEAS, PLI tements made herein of my of further that these statements or imprisonment, or both, up 126,916; Joseph 19, 100.	2,775; Peter D. Olexy, Reg. No. 24,835; Neil B. Siegel, R. Reg. No. 26,577; Sheldon E. 6,665; Alan J. Kasper, Reg. No. 30,951; Frank L. Bernstein anon, Reg. No. 32,778; Abratett S. Sylvester, Reg. No. 32. No. 32,607, Steven M. Grumy attorneys to prosecute this request that all corresponde JC, 2100 Pennsylvania Avenum knowledge are true and the work with the knowledge and Section 1001 of Title 1	No. 24,513; J. Frank Osh e.g. No. 25,200; David J. I. Landsman, Reg. No. No. 25,426; Kenneth J. I. Reg. No. 31,484; Marcham J. Rosner, Reg. No. 765; Robert M. Master askin, Reg. No. 36,818, is application and to training a policy of the application. N.W., Washington, I. that all statements made dge that willful false states of the United States.	ia, Reg. No. 24,625; Cushing, Reg. No. 25,430; Richard C. Burchfiel, Reg. No. rk Boland, Reg. No. o. 33,276; Bruce E. rs, Reg. No. 35,603, Peter A. McKenna, insact all business in the addressed to D.C. 20037-3213. on information and tements and the like
willful false statements may jeo				ОІИО
Date	r irs	t Inventor Tasuku First Name		ast Name
Residence Kyoto	Japan	Signature	五人	<u> </u>
City	State/Country			
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Citizenship Japanese				

Ďate		~~~~		Second Invente				TASHIRO
Residence	Kyoto		Japan C	Sig	nature	st Name Lei	Middle Initial Lors Peiro	Last Name
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Citizenship	Japanese					•		
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Mailing Addr	ress:	151 Beaume	ont Avenue, Rea	ding, MA 0186	7			
Citizenship	U.S.							
Date				Fifth Inventor	∑J _o	hn M. st Name	Middle Initial	McCoy Last Name
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Date				Sixth Inventor		sa A.	Middle Initial	Collins-Racie
Residence	Acton, MA		U.S.A. State/Country	Sign	nature		Pridate Initial	Last trame
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Citizenship	U.S.						Tank and a second	

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Date			Seventh Inventor		14.11.1.1.1	LaVallie
Residence	Harvard, MA City	U.S.A State/Country	Signa	First Name ture	Middle Initial	Last Name
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Citizenship						
Date Seca	mber 03, 20		Eighth Inventor	Maurice S	Middle Initial	Treacy Last Name
Residence	Dun Laoghair County of Dublin	re, IRELAND	Signa	A /	uk Xeex	D
Mailing Addı	City ress: 38 Clar	State/Country inda Park East, Dun	Laoghaire County	Dublin, IRELAN	D It	-
334						
Citizenship	IRELAND					
Date			Ninth Inventor	Cheryl First Name	Middle Initial	Evans Last Name
Residence	Germantown, MD City	U.S.A. State/Country	Signa Signa	. .		
Mailing Addi	Tace: 10326 (Golden Meadow Dri	ve, Germantown, N	MD 20876		
naming Addi	19320	Jordon Moddow Bir			-	
Citizenship	United Kingdom					
	United Kingdom			Michael J.		Agostino
Citizenship	United Kingdom			Michael J. First Name		Agostino Last Name
Citizenship Date Residence	United Kingdom Andover, MA City	U.S.A.	Tenth Inventor	Michael J. First Name	Middle Initial	Agostino Last Name
Citizenship Date	United Kingdom Andover, MA City	U.S.A. State/Country	Tenth Inventor	Michael J. First Name	Middle Initial	Agostino Last Name
Citizenship Date Residence Mailing Addi	United Kingdom Andover, MA City ress: 26 Wol	U.S.A. State/Country	Tenth Inventor	Michael J. First Name	Middle Initial	Agostino Last Name
Citizenship Cate Residence Mailing Addi	United Kingdom Andover, MA City ress: 26 Wol U.S.	U.S.A. State/Country cott Avenue, Andov	Tenth Inventor Signa er, MA 01810 Eleventh	Michael J. First Name ture Zhijian First Name	Middle Initial	Agostino Last Name
Citizenship Cate Residence Mailing Addi Citizenship Date	United Kingdom Andover, MA City ress: 26 Wol U.S. Bedford, MA City	U.S.A. State/Country cott Avenue, Andov	Tenth Inventor Signa er, MA 01810 Eleventh Inventor Signa	Michael J. First Name ture Zhijian First Name	Middle Initial	Agostino Last Name

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Ďate			Twelfth Inventor	David		Merberg
Residence	Acton, MA City	U.S.A. State/Country	Signatu	First Name	Middle Initial	Last Name
Mailing Addre	ess:	2 Orchard Drive, Acton, MA	01720			
	-					
Citizenship	U.S.					

DECLARATION AND POWER OF ATTORNEY

I/We as below named inventors, I/We hereby declare that our residence, mailing addresses and citizenship are as stated below next to my name: that verily believe I/We are the original, inventors (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter claimed and for which a patent is sought in the application entitled:

			THE POLYPEPTIDE	E AND UTILIZATION T	HEREOF
Which application		2 5 2002 55	F71 A		70
	tached application	2 5 2002		oplication No. <u>09/674,3</u> October 30, 2000 as the U	
(for original app	lication) JAN			JP99/02284, filed April 28	
	E E	Ø		71 7 77 0 E E O 1, 111 0 0 1 1 p 1 1 2 0	,, , , , , , , , , , , , , , , , , , , ,
	47D,	DEMARKE	(for d	eclaration not accompany	ing application)
I/We have revie	wed and understand the	contents of the speci	fication of the above-	identified application, inc	cluding the claims, as
amended by any	amendment referred to a	above; that I acknowled	ige my duty to disclose	e information of which I a	im aware and which is
				by claim priority benefits	
				ntor's certificate, §119(e)	
				gnated at least one country on for patent or inventor'	
	al application having a fi				s certificate of of any
1 C1 memation	a application having a m	ing date before that of	me application on wind		
Application ?	Number	Country	Filing Date	Priorit Yes	y Claimed No
Hei. 10-11		Japanese	April 28, 1998	<u></u>	
1161. 10-11	9151	Japanese	April 20, 1990		- Land
International application is no paragraph of Tit this application a	olication designating the of disclosed in a listed le 35, United States Cod	United States, listed by prior United States or le, §112, I acknowledged. 1.56 which occurred be	elow and, insofar as t PCT International ap e my duty to disclose	States application(s), or the subject matter of each oplication in the manner any information material of the prior application an	of the claims of this provided by the first to the patentability of
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A	pplication No.	rı	ling Date	Status	
Darryl Mexic, R Waddell A. Bigg 28,703; John R. Turner, Reg. No 31,333; Gordon 32,197; William Kramer, Reg. No George F. Lehni Reg. No. 38,551 the Patent and SUGHRUE, M	eg. No. 23,063; Robert V gart, Reg. No. 24,861; Lo Inge, Reg. No. 26,916; b. 29,710; Howard L. Be Kit, Reg. No. 30,764; Su H. Mandir, Reg. No. 3: b. 33,725; Paul F. Neils, igk, Reg. No. 36,359, Jo and Edward F. Kenehai Trademark Office conne	V. Sloan, Reg. No. 22,7 buis Gubinsky, Reg. No. Joseph J. Ruch, Jr., Remstein, Reg. No. 25,60 isan J. Mack, Reg. No. 2,156; Brian W. Hanno Reg. No. 33,102; Brett hn T. Callahan, Reg. No. 128,962, my exted therewith, and recard the second se	75; Peter D. Olexy, Rec. 24,835; Neil B. Siege. eg. No. 26,577; Sheld 55; Alan J. Kasper, Rec. 30,951; Frank L. Bermon, Reg. No. 32,778; A. S. Sylvester, Reg. No. 32,607, Steven M. Autorneys to prosecut quest that all corresponding A. 2100 Pennsylvania A.	e. 19,292; Robert J. Seas, eg. No. 24,513; J. Frank Cel, Reg. No. 25,200; David In Landsman, Reg. No. 25,426; Kenneth Listein, Reg. No. 31,484; Mabraham J. Rosner, Reg. 32,765; Robert M. Mass Gruskin, Reg. No. 36,81 e this application and to the ordence about the application of the condence about the application and the condence about the condence about the condence are application and the condence are	osha, Reg. No. 24,625; d. J. Cushing, Reg. No. o. 25,430; Richard C. J. Burchfiel, Reg. No. Mark Boland, Reg. No. No. 33,276; Bruce E. ters, Reg. No. 35,603, 8, Peter A. McKenna, ransact all business in ation be addressed to 1, D.C. 20037-3213.
belief are believe so made are pur	ed to be true; and further	that these statements wisonment, or both, und	ere made with the kno er Section 1001 of Ti	nd that all statements madewledge that willful false state 18 of the United State ing thereon.	statements and the like
Date		First Ir	ventor <u>Tasuku</u>		HONJO
			First Name	Middle Initial 2	Last Name
		Japan	Signature	7511	<u> </u>
(City	State/Country		y ·	
Mailing Address	10_4 Obcani	i-cho, Iwakura, Sakyo-k	u Kvoto-shi KVOTO	606-0001 IAPAN	
aiiiig riddiess		, 1	, 12,000 311, 121 010	. 555 0001 011111111	
		·			
Citizenship	Japanese				

Date 🚉			1 1 81 919 18	Second Invent				TASHIRO
			_			st Name	Middle Initial	Last Name
esidence	Kyoto		Japan C.	Sig	gnature	Fer	Cartino	
	City		State/Country				,	
ailing Addr	-ecc-	93 Higash	iohno-cho, Koya	ıma Kita-ku Kı	oto-shi	K VOTO 603	8162 TADAN	
5		75, 111 <u>5</u> u 51	aoimo eno, reoya	ina, rena ku, rej	oto sin,	1010 003	-0102 JAI AIN	
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itizenship	Japanese						•	
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ate				Third Inventor		moyuki		NAKAMURA
			•			st Name	Middle Initial	Last Name
esidence	San Diego,	California		Sig	nature	7021	nohom	
	City		State/Country			1		
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aning Addr	ess:	7003, Palm	nira Dr., #5324, S	San Diego, CA	·		*	
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itiaanshi	In							
itizenship	Japanese							
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ate				Fourth Invento	or 🕢 Ke	enneth		JACOBS
		to to a constant of the consta				st Name	Middle Initial	Last Name
esidence	Reading, M	[A	U.S.A.	Sig	nature			
	City		State/Country					· · · · · · · · · · · · · · · · · · ·
lailing Addr	ess:	151 Beaun	nont Avenue, Rea	iding, MA 0186	57			
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itizenship	_U.S					*		
ate				Fifth Inventor	∑ 01=1	an M		MaCan
<u></u>				rith inventor	V101	nn M. st Name	Middle Initial	McCoy Last Name
esidence	Reading, M	A	U.S.A	Sio	nature			
	City		State/Country	518				
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ailing Addr	ess:	56 Howard	Street, Reading,	MA 01867		8		
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itizenship	U.K.							
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ate				Sixth Inventor		a A.		Collins-Racie
						t Name	Middle Initial	Last Name
sidence	Acton, MA		U.S.A.	Sig	nature			
	City		State/Country					
ailing Addre	ess.	124 School	Street, Acton, M	14 01720				
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timomal 1	II C							
itizenship	U.S.							

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Date			_ Seventh Invento			LaVallie
Residence	Harvard, MA	U.S.A	Signa	First Name	Middle Initial	Last Name
	City	State/Country				
Mailing Add	tress:	Ann Lee Road, Harvai	rd, MA 01452			
0						
Citizenship	U.S.					
Date			E: 1.1 F	Maurice		Treacy
Residence	Dun Laogh County of Dublin		Signa	First Name	Middle Initial	Last Name
	City	State/Country		/ ·		
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Citizenship	IRELAND					
1990 <u> </u>	11/29/01		Ninth Inventor	Cheryl		Evans
Residence	Germantown, MI	mP _{U.S.A.}	- Signa	First Name	Middle Initial	Last Name
	City	State/Country) TYDVWY	
Mailing Adda	ress: 1932	6 Golden Meadow Dri	ve, Germantown, N	MD 20876		
Citizenship	United Kingdom	I				
Date		·	Tenth Inventor	Michael J.		Agostino
Residence	Andover, MA	U.S.A.	Signa	First Name	Middle Initial	Last Name
	City	State/Country	Signal		,	
Mailing Addr	ress: 26 W	olcott Avenue, Andov	er, MA 01810			
						* *
Citizenship	U.S.					
Date			Eleventh	Zhijian		Lu
			Inventor	First Name	Middle Initial	Last Name
Residence	Bedford, MA City	U.S.A. State/Country	Signat	yre		
Mailing Addr	•	-	16 1 144 0170	^		
viaining Audr	ESS. 120 C	ld Burlington Road, E	seatora, MA 01730	U		
Citizenship	People's Rep. Of	China				

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Date -		Twelft	h Inventor	David		Merberg	
Residence	Acton, MA	U.S.A.	Signati	First Name	Middle Initial	Last Name	
	City	State/Country	Signati				
Mailing Addre	ess:	2 Orchard Drive, Acton, MA 01720					
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Citizenship	U.S.						

ate 7-0			Seventh Inventor	Edward R. First Name	Middle to tial	LaVallie Last Name
Residence	Harvard, MA	U.S.A State/Country	Signal	ture dura	- 1/ V	Valler
Aailing Addı	•	nn Lee Road; <u>Harva</u>	1008	MA		
Citizenship	U.S.		-			
Date			_ Eighth Inventor	Maurice First Name	Middle Initial	Treacy Last Name
Residence	Dun Laogh County of Dublin		Signa	ture /		
Mailing Add	City 38 CI	State/Country arinda Park East, Du		/ Dublin, IRELAN)	
Citizenship	IRELAND					
Date			Ninth Inventor	Cheryl First Name	Middle Initial	Evans Last Name
		****	Ciano			
Residence	Germantown, MI City	State/Country	Signa			
Residence Mailing Add	City					
	City	State/Country 6 Golden Meadow D				
Mailing Add	City ress: 1932 United Kingdom	State/Country 6 Golden Meadow D	rive, Germantown, I	MD 20876 Michael J. First Name (1)	∩Mid dl e Initial,	_Agostino Last Name
Mailing Add	City ress: 1932	State/Country 6 Golden Meadow D	rive, Germantown, I Tenth Inventor Signa	MD 20876 Michael J.	∩Mid dl e Initial,	Agostino
Mailing Add Citizenship Date	City ress: 1932 United Kingdom Andover, MA City	State/Country 6 Golden Meadow D 1 1 1 1 1 1 1 1 1 1 1 1 1	Tenth Inventor	MD 20876 Michael J. First Name (1)	∩Mid dl e Initial,	Agostino
Mailing Add Citizenship Date Residence	City ress: 1932 United Kingdom Andover, MA City	State/Country 6 Golden Meadow D 1 1 1 1 1 1 1 1 1 1 1 1 1	Tenth Inventor	MD 20876 Michael J. First Name (1)	∩Mid dl e Initial,	Agostino
Mailing Add Citizenship Date Residence Mailing Add	City ress: 1932 United Kingdom Andover, MA / City dress: 26 W	State/Country 6 Golden Meadow D 1 1 1 1 1 1 1 1 1 1 1 1 1	Tenth Inventor	MD 20876 Michael J. First Name Atture Zhijian	Middle Initial	Agostino Last Name Lu
Mailing Add Citizenship Date Residence Mailing Add Citizenship	City ress: 1932 United Kingdom Andover, MA / City dress: 26 W	State/Country 6 Golden Meadow D 1 1 1 1 1 1 1 1 1 1 1 1 1	Tenth Inventor Signationer, MA 01810 Eleventh Inventor Signation	MD 20876 Michael J. First Name Zhijian Eirst Name	∩Mid dl e Initial,	Agostino Last Name

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Date Residence Actor City		J.S.A. Twelfth State/Country	Inventor Davi First I Signature	Merberg le Initial Last Name Mercera
Mailing Address:	2 Orchard D	rive, Acton, MA 01720		
Citizenship				
JAN 25	2005 F.			
TRADE	MARINE			